



MERCK

# Scalability and Performance Guide

## Mobius® iFlex Bioreactors

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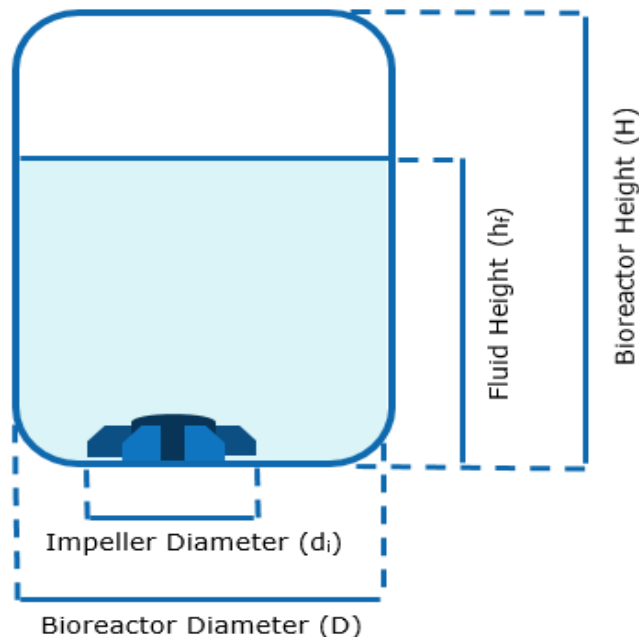
Millipore®

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## The Mobius® iFlex Single-use Bioreactor

Intensified bioprocessing arises from an increased need for high-quality drug supply while lowering facility footprints and production costs. Specifically, upstream processes are adopting intensified fed-batch and perfusion within single-use bioreactors to realize higher flexibility and increased product yields. The Mobius® iFlex bioreactor platform offers single-use, stirred tank systems designed to support both traditional fed-batch and perfusion intensified cell culture applications. Performance attributes of the impeller, spargers, Ultimus® film and sensors were characterized to determine the effective design space and operating range for accurate process monitoring and control. Scalability data developed in representative systems with volumes ranging from 50 L to 2000 L are also presented as a guide for process development and cross-scale performance expectations.



**Diagram 1:** *Bioreactor dimensions*

## Geometric Scalability

Bioreactor vessel design impacts performance parameters of mixing, gas mass transfer rate, power per volume and heat transfer. Therefore, to make bioreactors more easily scalable, vessel and component geometries were maintained across the 50 L to 2000 L bioreactors to simplify strategies for scale-up from pilot to commercial operations. The aspect ratio (H:D) of a bioreactor compares the inner vessel height to diameter geometry. The Mobius® iFlex bioreactors were designed with a constant 2:1 ratio, a common ratio for bioreactor mechanical designs to support cell culture processes.<sup>1</sup> The turndown ratio dictates the acceptable minimum working volume at each vessel scale. The Mobius® iFlex Bioreactors have high turndown ratios, increasing the flexibility for use and limiting the number of vessels needed in a single process and reducing facility footprint. The working volumes for the Mobius® iFlex Bioreactors range from 15 L to 2000 L for 50 L to 2000 L bioreactors.

## Design Characterization of Mobius® iFlex Bioreactors

**Table 1**

|                                 | 50 L*                                      | 200 L                                      | 500 L*                                     | 1000 L*                                     | 2000 L                                      |
|---------------------------------|--|--|--|---|---|
| H:D                             | 2.0:1                                      | 2.0:1                                      | 2.0:1                                      | 2.0:1                                       | 2.0:1                                       |
| Maximum Working Volume (L)      | 50   | 200  | 500  | 1000  | 2000  |
| Minimum Working Volume (L)      | 15   | 40   | 100  | 200   | 400   |
| $h_{f,max}:H$                   | 0.8:1                                      | 0.8:1                                      | 0.8:1                                      | 0.8:1                                       | 0.8:1                                       |
| $h_{f,min}:h_{f,max}$           | 3.3:1                                      | 5.0:1                                      | 5.0:1                                      | 5.0:1                                       | 5.0:1                                       |
| $d_i:D$                         | 0.34                                       | 0.38                                       | 0.35                                       | 0.34  | 0.35  |
| Vessel Diameter (cm)            | 34.0                                       | 54.6                                       | 72.9                                       | 91.9  | 115.8                                       |
| Impeller Diameter (cm)          | 11.7                                       | 21.1                                       | 25.4                                       | 31.1  | 40.6  |
| Blade Height (cm)               | 4.1  | 6.6  | 7.1  | 7.5   | 10.4  |
| Blade Sweep (deg)               | 18.0                                       | 13.5                                       | 11.0                                       | 11.0  | 11.0  |
| Blade angle (deg)               | 13.5                                       |  |  |   |   |
| Impeller Geometry               | Down-pumping pitched blade (4 blades)      |  |  |   |   |
| Impeller Position               | Bottom mount, 15° from center              |  |  |   |   |
| Impeller Power Number ( $N_p$ ) | 3.6  | 3.6  | 3.6  | 3.6   | 3.7   |
| Baffles                         | X - baffle                                 |  |  |   |   |
| Open Pipe Sparger Orifice (mm)  | 4.4  | 7.4  | 2x 7.4                                     | 2x 10.4                                     | 2x 10.4                                     |
| Mid-range Drilled-hole Sparger  | 952 150 $\mu$ m drilled-holes in Ultimus®  | 2357 150 $\mu$ m drilled-holes in Ultimus® | 4714 150 $\mu$ m drilled-holes in Ultimus® | 7071 150 $\mu$ m drilled-holes in Ultimus®  | 9428 150 $\mu$ m drilled-holes in Ultimus®  |
| High Performance Sparger        | 15912 25 $\mu$ m drilled-holes in Ultimus® | 36216 25 $\mu$ m drilled-holes in Ultimus® | 72432 25 $\mu$ m drilled-holes in Ultimus® | 108648 25 $\mu$ m drilled-holes in Ultimus® | 144864 25 $\mu$ m drilled-holes in Ultimus® |

\*In development

# Impeller, Baffle and Mixing Performance

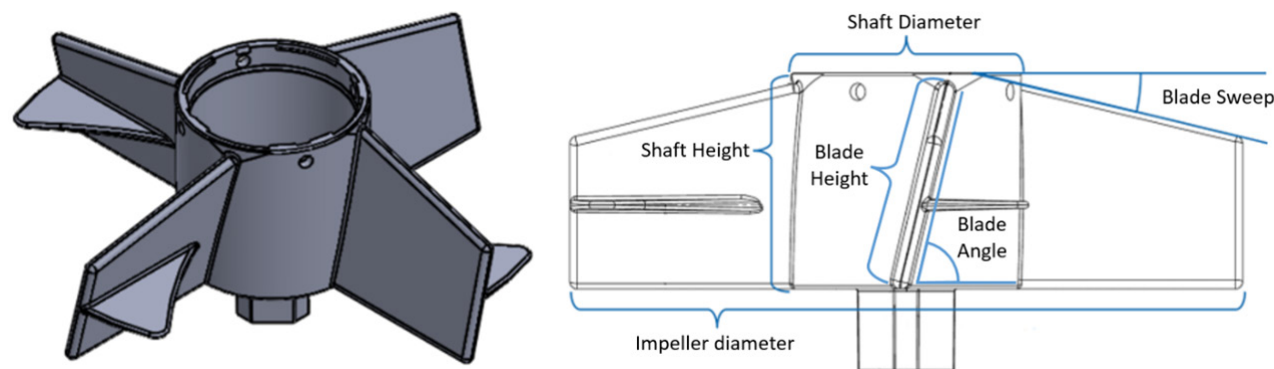
## Power Density

Power per unit volume describes the energy transfer from the mechanical mixing into cell culture medium in the bioreactor system. Effective scale-up to 2000 L is highly dependent on achieving constant power density per system as it enables homogeneous mixing and optimized mass transfer. To calculate power density and scale up, impeller power number ( $N_p$ ) is used. Power number is a proportionality constant between the rotational velocity of an impeller and the amount of energy it imparts into the mixing fluid and should be constant across systems for scalability (Eq. 1-3)<sup>2,3</sup> (refer to [Equations](#) for more information). Power number is unique to the impeller, motor, and vessel designs, and can be impacted by baffles and proximity to walls within the vessel. In the Mobius® iFlex bioreactors, impellers were designed to maintain a constant power number across all system sizes for scalable power input, mixing, and mass transfer performance.

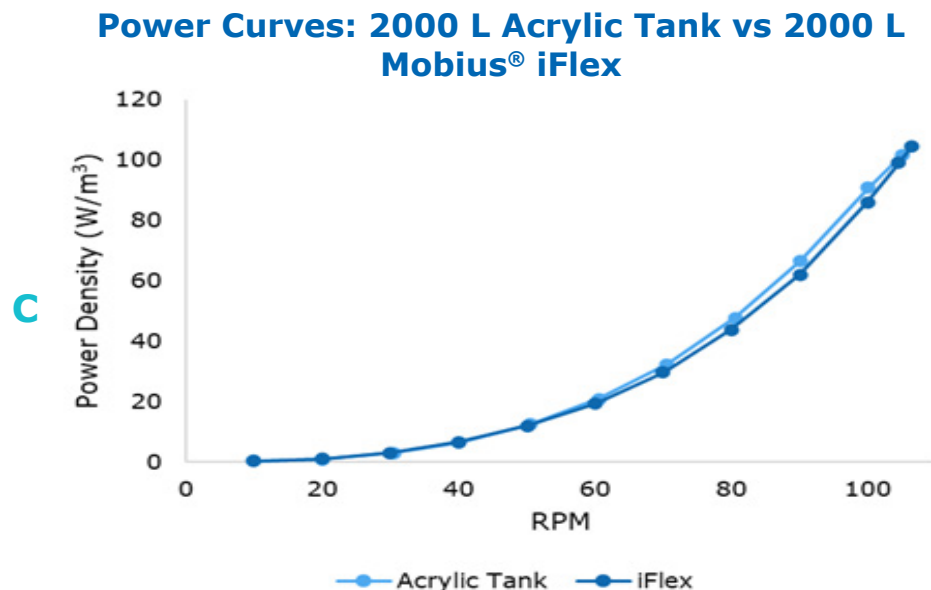
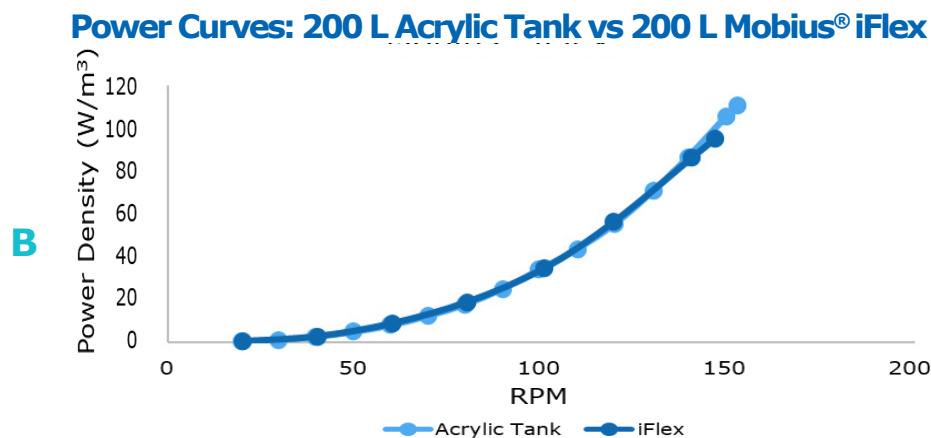
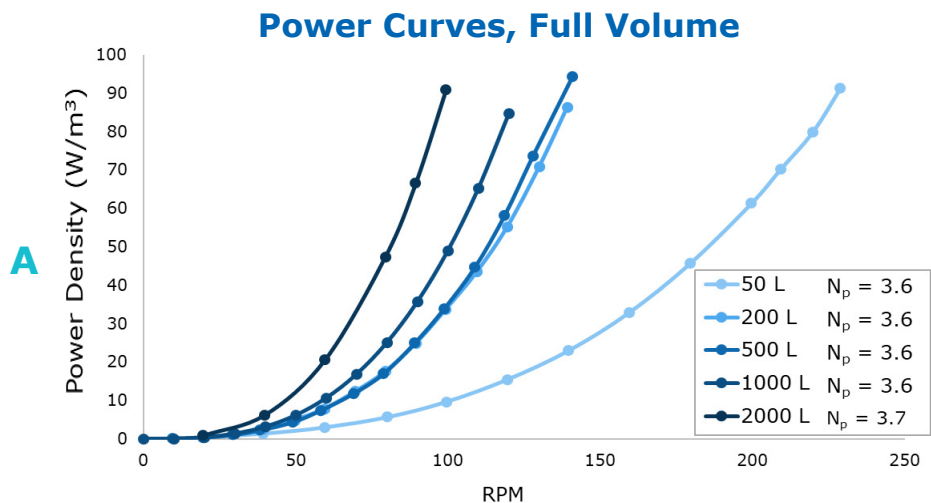
For 50, 200, 500, 1000 and 2000 L sizes, acrylic tank systems were constructed with exact dimensions of the bioreactor systems including associated motor designs per scale. Using software unique to the motor, drive current usage was measured at various RPMs. For 20 seconds per RPM, the average drive current value was obtained. Torque was calculated from drive current based on conversions from the motor manufacturers specifications. The average recorded RPM for each setpoint was converted into an angular velocity and combined with torque to determine the power required to turn the impeller in liquid.

The reported power number for each impeller design was calculated as the average of all power numbers obtained at RPM setpoints corresponding to turbulent Reynold's numbers above at least 50,000 up to the maximum RPM of the motor. Power number was calculated at full volume with water at ambient temperature, without sparging.

Impeller stability was also considered in the design by adding perpendicular fins to the blades to ensure stability across the entire RPM range. This was assessed using the motor software to monitor a dimensionless tilting parameter, which showed reduced tilt with little effect on power number or mixing performance with the addition of the perpendicular fins.<sup>4</sup>



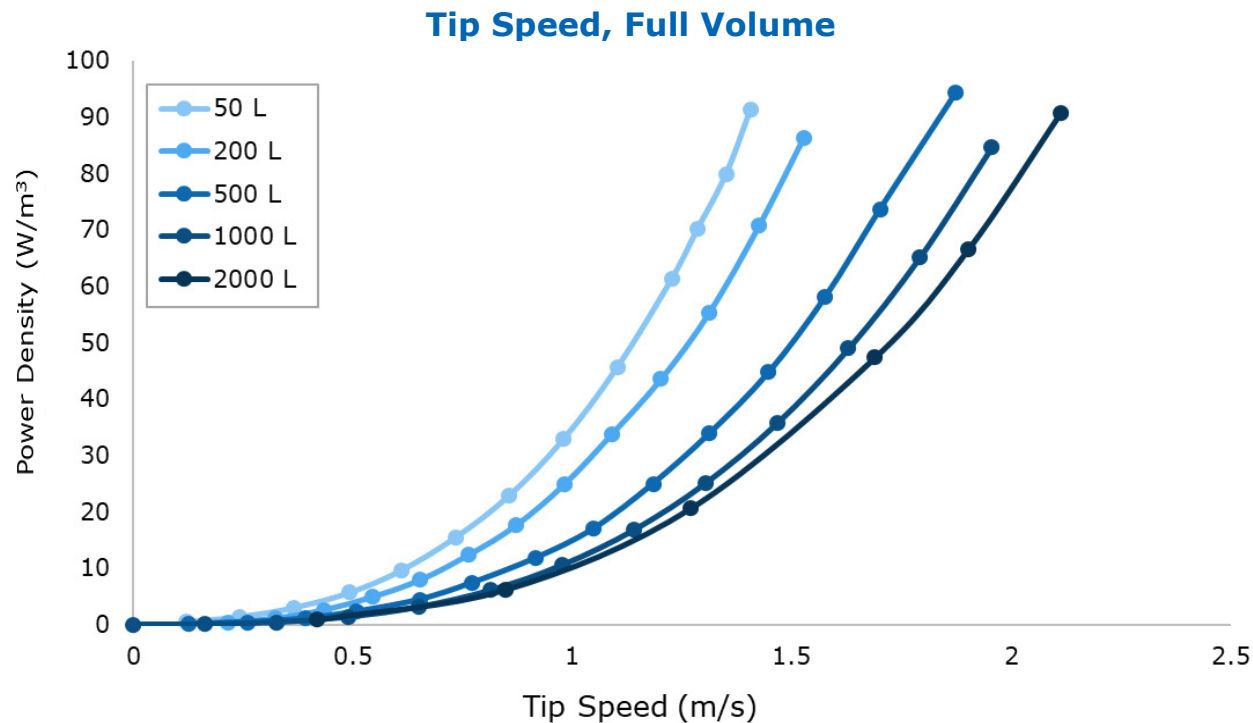
**Diagram 2:** *Impeller design dimensions optimized for consistent  $N_p$*



**Figure 1:** (A) Power per unit volume ( $W/m^3$ ) curves for a range of impeller RPMs at 50 L to 2000 L scales in representative acrylic tanks. (B) Power curve comparison for 200 L acrylic tank data vs. 200 L Mobius® iFlex system. (C) Power curve comparison for 2000 L acrylic tank data vs. 2000 L Mobius® iFlex system. Data is shown at full volume for  $n=1$  runs averaging at least  $n=20$  drive current points per RPM. Power number ( $N_p$ ) is calculated using Eq. 4.

## Tip Speed and Shear Rate

Tip speed, an important parameter associated with shear, was calculated based on the maximum RPM required to reach  $100 \text{ W/m}^3$  at each scale up to 2000 L. For all scales, multiple impeller iterations were evaluated to deliver a final design where the target power number ( $N_p$ ) could be reached, while maintaining less than or equal to  $2.2 \text{ m/s}$  tip speed at a maximum of  $100 \text{ W/m}^3$ . Meeting these criteria support cross system scalability and reduce shear risk associated with agitation.<sup>5</sup>



**Figure 2:** Power per unit volume ( $\text{W/m}^3$ ) curves at a range of impeller tip speeds for 50 L to 2000 L scales. Data is shown at full volume for  $n=1$  runs averaging at least  $n=20$  drive current points per RPM. Tip speed is calculated using Eq. 6.

Table 2

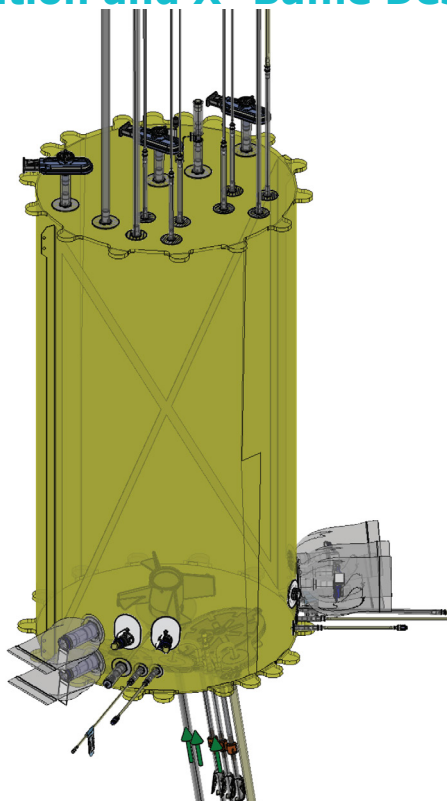
| Bioreactor Scale                 | Volume (L)        | P/V (W/m <sup>3</sup> )<br>Eq. 1 | RPM | Tip Speed (m/s)<br>Eq. 6 | Re Eq. 5 | Tip Shear Rate (1/s)<br>Eq. 7 | Cup Shear Rate (1/s)<br>Eq. 8 |
|----------------------------------|-------------------|----------------------------------|-----|--------------------------|----------|-------------------------------|-------------------------------|
| 50 L<br>(N <sub>p</sub> : 3.6)   | Minimum<br>(15)   | 10                               | 74  | 0.45                     | 19,000   | 22.8                          | 209                           |
|                                  |                   | 50                               | 126 | 0.77                     | 32,000   | 39.0                          | 357                           |
|                                  |                   | 100                              | 158 | 0.97                     | 40,000   | 49.1                          | 450                           |
|                                  | Maximum<br>(50)   | 10                               | 110 | 0.68                     | 28,000   | 34.1                          | 312                           |
|                                  |                   | 50                               | 188 | 1.16                     | 48,000   | 58.2                          | 534                           |
|                                  |                   | 100                              | 237 | 1.46                     | 61,000   | 73.4                          | 673                           |
| 200 L<br>(N <sub>p</sub> : 3.6)  | Minimum<br>(40)   | 10                               | 39  | 0.43                     | 32,000   | 12.9                          | 185                           |
|                                  |                   | 50                               | 67  | 0.73                     | 54,000   | 22.0                          | 316                           |
|                                  |                   | 100                              | 84  | 0.92                     | 69,000   | 27.7                          | 399                           |
|                                  | Maximum<br>(200)  | 10                               | 67  | 0.73                     | 54,000   | 22.0                          | 316                           |
|                                  |                   | 50                               | 114 | 1.25                     | 93,000   | 37.7                          | 541                           |
|                                  |                   | 100                              | 144 | 1.58                     | 117,000  | 47.4                          | 682                           |
| 500 L<br>(N <sub>p</sub> : 3.6)  | Minimum<br>(100)  | 10                               | 38  | 0.51                     | 46,000   | 14.3                          | 122                           |
|                                  |                   | 50                               | 66  | 0.87                     | 78,000   | 24.4                          | 209                           |
|                                  |                   | 100                              | 83  | 1.10                     | 99,000   | 30.8                          | 263                           |
|                                  | Maximum<br>(500)  | 10                               | 66  | 0.87                     | 78,000   | 24.4                          | 209                           |
|                                  |                   | 50                               | 112 | 1.49                     | 134,000  | 41.8                          | 357                           |
|                                  |                   | 100                              | 141 | 1.88                     | 169,000  | 52.6                          | 450                           |
| 1000 L<br>(N <sub>p</sub> : 3.6) | Minimum<br>(200)  | 10                               | 34  | 0.56                     | 62,000   | 14.7                          | 154                           |
|                                  |                   | 50                               | 59  | 0.96                     | 106,000  | 25.2                          | 264                           |
|                                  |                   | 100                              | 74  | 1.21                     | 133,000  | 31.7                          | 333                           |
|                                  | Maximum<br>(1000) | 10                               | 59  | 0.96                     | 106,000  | 25.2                          | 264                           |
|                                  |                   | 50                               | 101 | 1.64                     | 180,000  | 43.0                          | 451                           |
|                                  |                   | 100                              | 127 | 2.07                     | 227,000  | 54.2                          | 569                           |



| Bioreactor Scale                 | Volume (L)        | P/V (W/m <sup>3</sup> ) Eq. 1 | RPM | Tip Speed (m/s) Eq. 6 | Re Eq. 5 | Tip Shear Rate (1/s) Eq. 7 | Cup Shear Rate (1/s) Eq. 8 |
|----------------------------------|-------------------|-------------------------------|-----|-----------------------|----------|----------------------------|----------------------------|
| 2000 L<br>(N <sub>p</sub> : 3.7) | Minimum<br>(400)  | 10                            | 28  | 0.59                  | 85,000   | 11.3                       | 124                        |
|                                  |                   | 50                            | 47  | 1.01                  | 145,000  | 19.3                       | 213                        |
|                                  |                   | 100                           | 60  | 1.27                  | 183,000  | 24.3                       | 268                        |
|                                  | Maximum<br>(2000) | 10                            | 47  | 1.01                  | 145,000  | 19.3                       | 213                        |
|                                  |                   | 50                            | 81  | 1.72                  | 248,000  | 32.9                       | 363                        |
|                                  |                   | 100                           | 102 | 2.17                  | 312,000  | 41.5                       | 458                        |

Summary of power input, tip speed and shear results at maximum and minimum volumes for 50 L to 2000 L vessels. Tip shear rate is associated with impeller diameter. Cup shear rate is associated with the impeller and motor design, describing the part of the bioreactor assembly that resembles a cup, and contains the magnetic portion of the impeller assembly which couples with the motor and houses the base of the impeller as it levitates during rotation.

## Impeller Location and X- Baffle Design



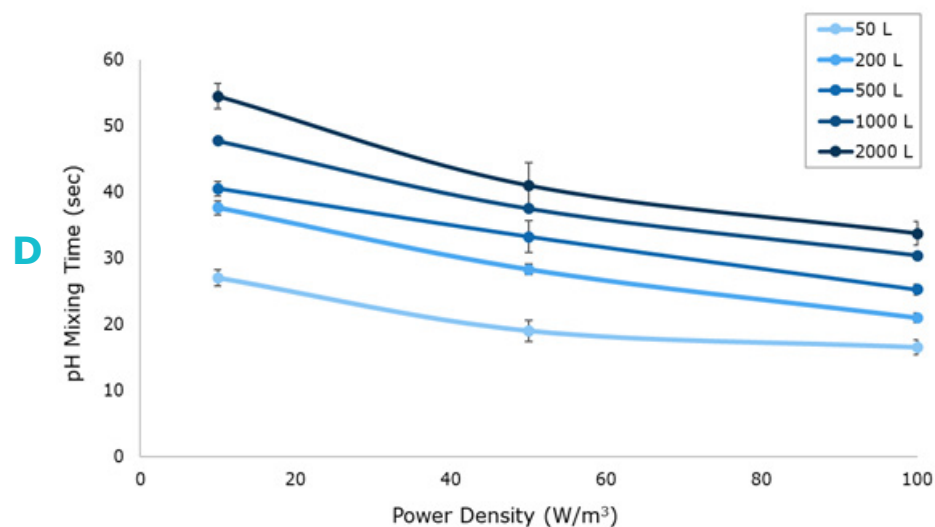
**Diagram 3:** X-baffle design within the 200 L single-use bag design.

The impeller is bottom-mounted and position off-center in the base of the bioreactor assembly at a 15 degree angle above horizontal. To further prevent vortices and improve mixing efficiency, an X-baffle design is incorporated into the single-use bag assembly. Various baffle designs were tested for mixing (not shown) within previous systems and the X-shape was chosen as the optimal solution for minimized mixing time and maximized mixing homogeneity. The design and orientation of the X-baffle was optimized by assessing power number at different locations in relation to the impeller, and was confirmed with mixing studies.<sup>4</sup>

## Mixing Time

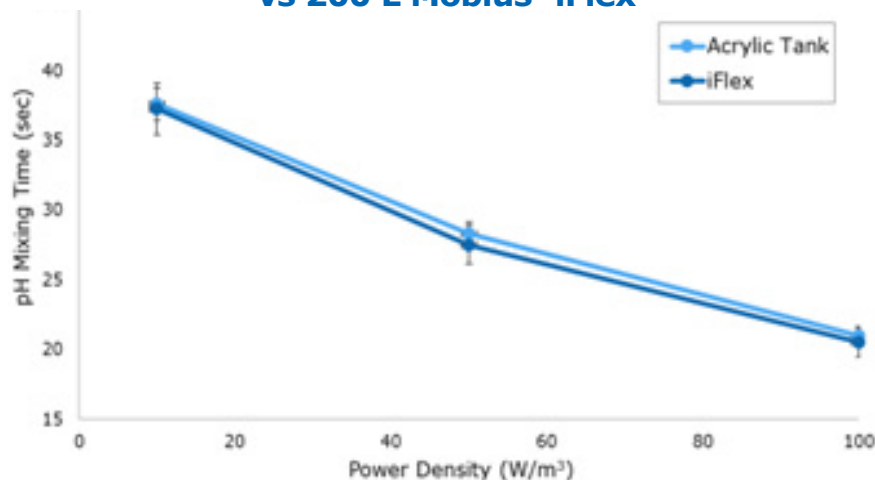
To assess the mixing performance, experiments were conducted at maximum working volumes and power densities ranging from 10-100 W/m<sup>3</sup> in representative acrylic tank systems. Mixing times determined in acrylic tanks were also confirmed in final Mobius® iFlex systems for 200 and 2000 L scales. Optimized methods of determining mixing times were compared by several sensor and colorimetric mixing methods typical in industry. The use of a pH sensor mixing method and a phenolphthalein colorimetric method were determined fit for characterization based on accuracy, utility, and ease of use.<sup>6</sup> For the pH method, pH was adjusted to 4.0 at the start of the experiment using DI water and HCl. Two pH probes were placed within the tank and response curves were recorded following the addition of concentrated acid or base. For the 50 L scale, only one pH probe was used due to space constraints in the acrylic system. Solutions of 5M NaOH and HCl were alternatively added to the center of the tank once stability was reached at a ratio of 1 mL per 25 L of solution. Mixing time was determined as the time for the pH profile to reach 99% of the final resting value at each agitation rate tested. For the colorimetric method, mixing time was measured visually from the time of addition until the last trace of pink color disappeared.

### pH Mixing Time, Full Volume



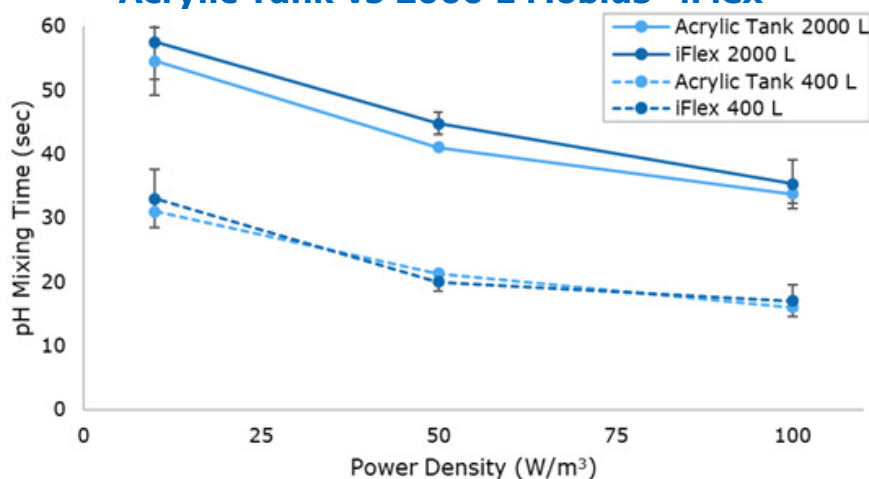
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**pH Mixing Time, Full Volume: 200 L Acrylic Tank vs 200 L Mobius® iFlex**

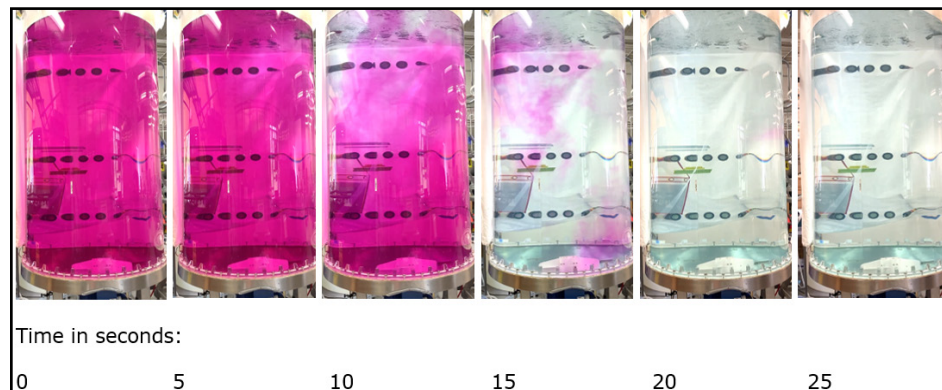


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**pH Mixing Time, Min & Max Volume: 2000 L Acrylic Tank vs 2000 L Mobius® iFlex**



**Figure 3:** (D) pH mixing time for 10, 50 and 100 W/m<sup>3</sup> power input respective to vessel size from 50 L to 2000 L in representative acrylic tanks. (E) Mixing time comparison for 200 L acrylic tank data vs. 200 L Mobius® iFlex system. (F) Mixing time comparison for 2000 L acrylic tank data vs. 2000 L Mobius® iFlex system. Data is shown as the average ± SD at full volume and minimum volume for the 2000 L scale for n = 4 runs.



**Diagram 4:** Phenolphthalein colorimetric mixing example at full volume in the 2000 L acrylic tank with the X- baffle and a power input of 100 W/m<sup>3</sup>.

## Duration Study

A continuous duration study was completed on the Mobius® iFlex 200 L and 2000 L bioreactor. The flexware was connected to the perfusion tower to mimic the perfusion process and both the drilled hole and high performance sparger were used at nominal gas flow rates for 40 days. Pumps were run with nominal settings. The bioreactor was filled to maximum volume with water and heated to 37°C at the start of the test. The impeller was operated at 114 RPM (50 W/m<sup>3</sup>) for the 200 L scale and at 81 RPM (50 W/m<sup>3</sup>) for the 2000 L scale continuously for 40-days and showed no issues during testing.

## Summary

- Impeller designs were optimized for shear and scalability considerations. All scales allow for up to 100 W/m<sup>3</sup> with <35 second mixing time while maintaining a tip speed of under 2.2 m/s for a single impeller.
- Appropriate impeller shaft diameter, blade sweep, height, impeller diameter, and stabilizing fins, along with an optimized baffle design gives a constant power number of 3.6 to 3.7 across scales.
- Impeller design is robust as demonstrated by a 40-day continuous duration.

# Sparger Performance

## Increased Capacity and Flexibility

To support intensified processes, the sparging system relating to oxygenation performance is critical. With higher viable cell density, there is increased demand for oxygen. Expanding the process window for oxygenation performance required to support intensified processes is achieved through the design of optimized impeller and sparger components in the bag assembly, as well as providing higher torque motors and high-capacity mass flow controllers as part of the hardware offering at each scale (refer to [Table 4](#) for more information). The Mobius® iFlex sparging system offers maximum flexibility to meet a wide range of process needs, as each single-use assembly includes 3 spargers: an open pipe sparger with 4400-10400  $\mu\text{m}$  holes (open pipe), a mid-range drilled-hole sparger with 150  $\mu\text{m}$  holes (drilled-hole), and a drilled-hole sparger with 25  $\mu\text{m}$  holes (high performance).

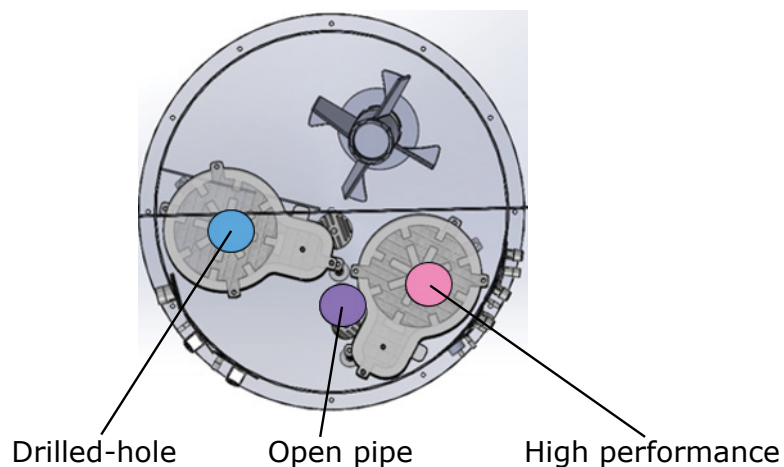
Pore size and use cases of the open pipe, drilled-hole and high-performance sparger options are shown in table 3. Theoretical VCD capacity is estimated based on a CHO cell line that has mid/high oxygen consumption rate ( $q\text{O}_2$ ) using  $k_La$  determined in Mobius® iFlex systems. Higher VCD is expected to be supported with lower  $q\text{O}_2$  values.

**Table 3**

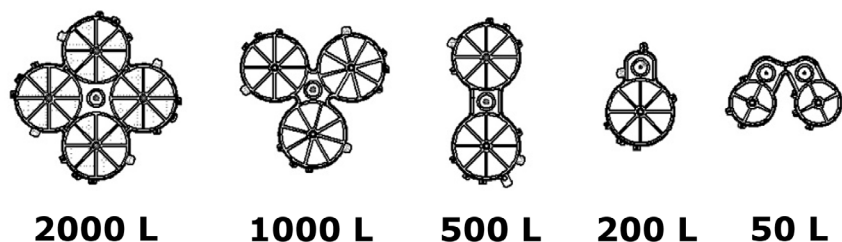
| Sparger          | Pore Size ( $\mu\text{m}$ ) | Purpose  | Theoretical VCD Capacity based on $q\text{O}_2^*$ |
|------------------|-----------------------------|--|---|
| Open pipe        | 4400 - 10400                | CO <sub>2</sub> stripping and oxygenation, low shear risk        | 20 e6 cells/mL                                    |
| Drilled-hole     | 150                         | CO <sub>2</sub> stripping and oxygenation, low to mid shear risk | 120 e6 cells/mL                                   |
| High performance | 25                          | Oxygenation, mid to high shear risk                              | 200 e6 cells/mL**                                 |

\* $q\text{O}_2$  = cell specific oxygen uptake rate of 5 pmol/cell/day (mid-high value for CHO cells<sup>2</sup>): using 100% oxygen, 100 W/m<sup>3</sup> and 40% MFC capacity based on 200 L  $k_La$  calculated from Eq. 11.

\*\*High performance sparger utilizes 1X PBS  $k_La$  which was found to be more representative of HD Perfusion Media for that sparger.



**Diagram 5:** *Sparger locations at bottom of 200 L bag*



**Diagram 6:** *Drilled-hole and high performance sparger configurations for various vessel sizes.*

**Note** Each scale will have 2 separate spargers with the above configuration at the bottom of the bag, with the exception of the 50 L scale. The 50 L scale will have 1 of the displayed configuration, with the high performance sparger on one lobe and the drilled hole on the second.

## Velocity and Gas Distribution

Theoretical entrance velocity from the sparger to the cell culture media is an important parameter associated with shear, gas distribution and  $k_L a$  performance. As such, entrance velocity was used as a scaling parameter in designing the Mobius® iFlex Bioreactor spargers. Entrance velocity is considered shear safe below  $<60$  m/s for CHO cell lines.<sup>8</sup> For spargers, the open area is calculated by the open pipe diameter or size and number of holes in a drilled-hole sparger according to Eq. 9. The open area of the sparger is then linearly scaled based on the maximum flow rate of the sparger to keep entrance velocity consistent per scale and per sparger pore size. To minimize risk, the open pipe and drilled-hole spargers were designed to produce up to 22 and 20 m/s entrance velocity at the maximum flow rate recommended through each sparger type. The high performance sparger is designed as a tool to push the limits of oxygenation in the system, meeting an entrance velocity up to 19, 38 and 47 m/s at 40, 80, 100% of the recommended maximum flow rate through the sparger, respectively.

**Table 4**

Standard mass flow controller (MFC) gas flow range for each bioreactor scale and gas option:

| MFC  | Gas   | Core/Option | SLPM      |          |           |           |            |
|------|---|-------------|-----------|----------|-----------|-----------|------------|
|      |   |             | 50 L      | 200 L    | 500 L     | 1000 L    | 2000 L     |
| MFC1 | Air   | Core        | 0.08 - 20 | 0.2 - 50 | 0.4 - 100 | 0.6 - 150 | 16.7 - 200 |
| MFC2 | O <sub>2</sub> (but calibration table can be selected and changed to Air) | Core        | 0.08 - 20 | 0.2 - 50 | 0.4 - 100 | 0.6 - 150 | 16.7 - 200 |
| MFC3 | CO <sub>2</sub>   | Core        | 0.08 - 15 | 0.2 - 37 | 0.2 - 37  | 0.2 - 37  | 0.2 - 37   |
| MFC4 | N <sub>2</sub>  | Core        | 0.08 - 20 | 0.2 - 50 | 0.2 - 50  | 0.2 - 50  | 0.2 - 50   |
| MFC5 | Air   | Option      | 0.08 - 20 | 0.2 - 50 | 0.2 - 50  | 0.2 - 50  | 0.2 - 50   |
| MFC6 | O <sub>2</sub>  | Option      | 0.08 - 20 | 0.2 - 50 | 0.2 - 50  | 0.2 - 50  | 0.2 - 50   |
| MFC7 | N <sub>2</sub> (other gas can be selected)                                | Option      | 0.08 - 20 | 0.2 - 50 | 0.2 - 50  | 0.2 - 50  | 0.2 - 50   |

**Note** MFC operating gas range and calibration of Air, O<sub>2</sub>, CO<sub>2</sub>, N<sub>2</sub> can be selected through Brooks Service Software or through Ethernet IP connection to the MFCs. MFC4-7 are configurable, therefore the ranges listed are dependent on the MFC selection.

**Table 5**

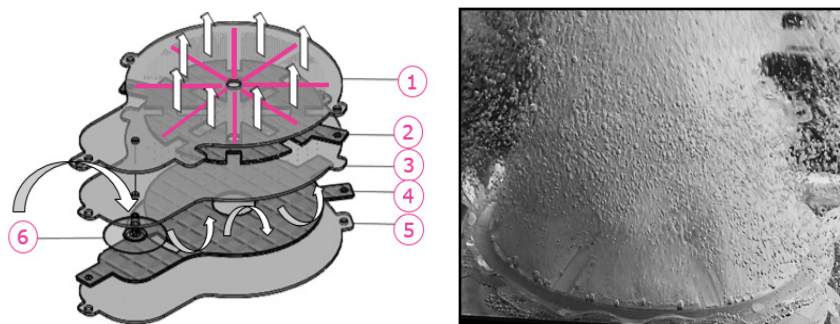
Sparger outlet maximum capacity for 50 L to 2000 L scales and theoretically derived entrance velocity (m/s) for each sparger design:

| Scale (L) | Maximum Flow Rate through Sparger (SLPM) | Open Pipe (m/s) | Drilled-hole Sparger (m/s) | High Performance Sparger Flow Rate (m/s) |      |
|-----------|--|-----------------|----------------------------|--|------|
|           |  |                 |                            | 40%*                                     | 100% |
| 50        | 20                                       | 22              | 20                         | 17                                       | 43   |
| 200       | 50                                       | 19              | 20                         | 19                                       | 47   |
| 500       | 100                                      | 19              | 20                         | 19                                       | 47   |
| 1000      | 150                                      | 15              | 20                         | 19                                       | 47   |
| 2000      | 200                                      | 20              | 20                         | 19                                       | 47   |

\*Typical usage of high-performance sparger is expected to be at 40% capacity, or below maximum flow capacity.

Drilled-hole spargers were designed with an innovative gas distribution method. This allows for all the drilled-holes to be utilized even at lower flow rates and at the 15° tank angle for improved volumetric mass transfer.

1. Drilled-hole sheet with specified velocity.
2. Mesh layer for support.
3. Drilled-hole sheet to build back pressure.
4. Mesh layer for support.
5. Bottom layer.
6. Gas inlet port.



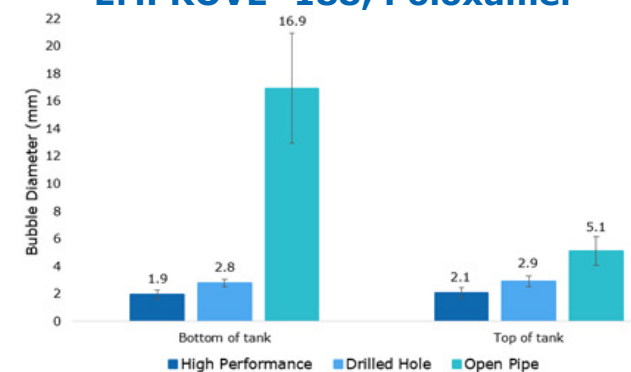
**Diagram 7:** Layered components of the drilled-hole sparger for even gas distribution.

## Bubble Size

Bubble size is an important parameter of mass transfer performance and bubble shear on cells. Bubble shear is caused by bubbles bursting at the surface, where small bubbles (<2 mm) have higher energy at burst leading to higher risk of cell damage.<sup>9,10</sup> To protect cells against bubble shear, poloxamers are typically included in bioreactor media, which decrease surface tension and cause less damage to cells at burst. Due to the importance of bubble size, spargers were designed to maintain a constant bubble size to stay within shear limits from the drilled-hole material with consistent pore size. The offering of multiple sparger types with different bubble sizes gives options based on the bubble shear sensitivity of the cell line.

To measure bubble size and design sparger pore sizes, a 200 L representative acrylic tank with spargers, a baffle, and an impeller was used. Bubble size was measured at the top and bottom of the tank at various process conditions. Media was imitated by a solution of Dulbecco Phosphate Buffered Saline (1X PBS) and 4 g/L Poloxamer 188 EMPROVE® EXPERT. A high-speed camera captured bubbles created by the sparger within the same frame as a reference measurement. Using the software ImageJ, frames were converted into 7-10 images, and bubbles were traced in the same plane as the reference measurement within the software. 50 to 100 bubbles were traced per condition and ImageJ output the minimum and maximum diameter of bubbles and converted to circular bubble diameter through Eq. 10. Average bubble diameter and standard deviations were then calculated for each condition.

## 200 L Spargers Bubble Size 20 SLPM 20 W/m<sup>3</sup>, 1X PBS 4 g/L EMPROVE® 188, Poloxamer



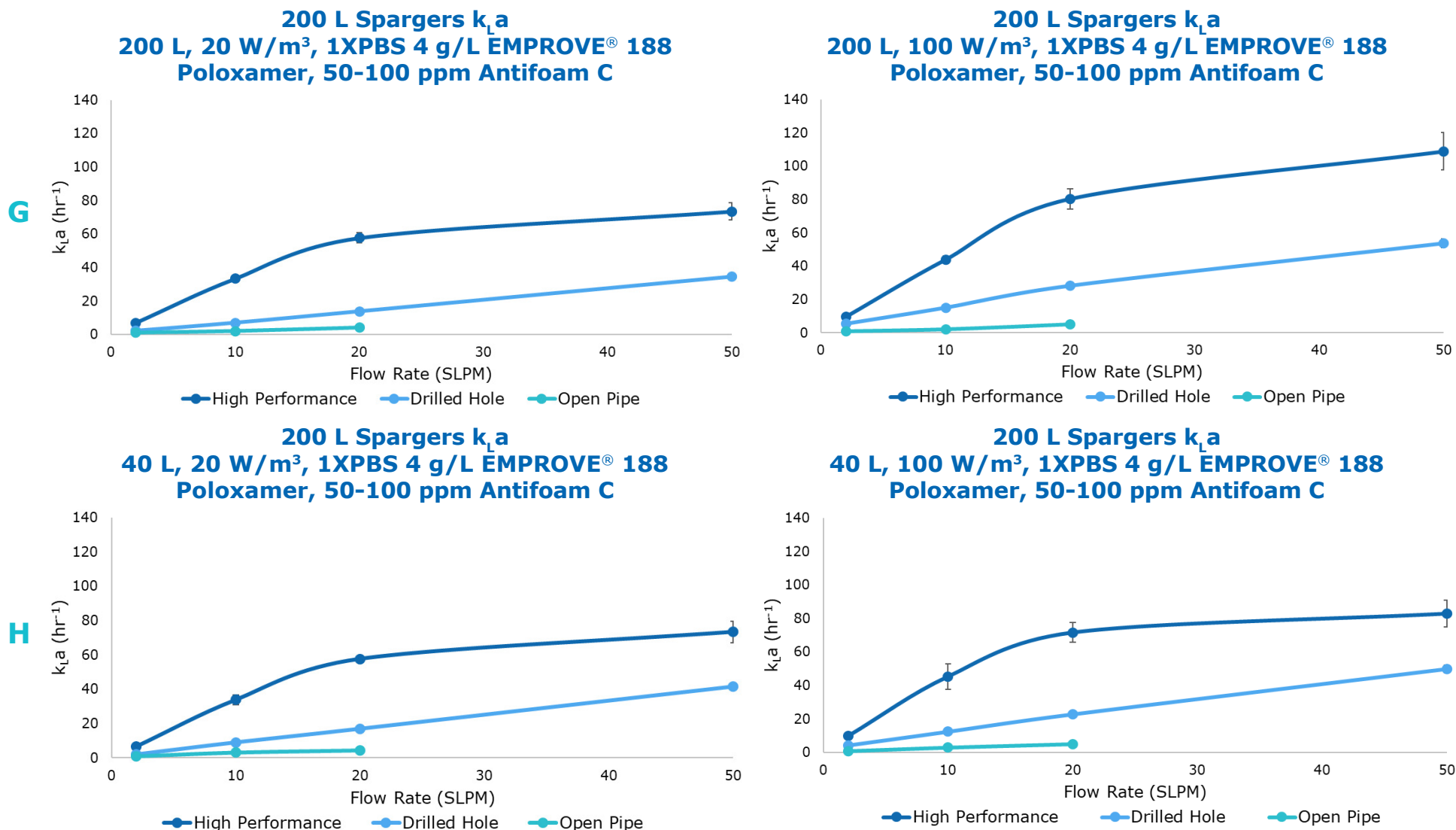
**Figure 4:** Bubble size analysis for high performance, drilled-hole and open pipe spargers at 200 L scale, 20 W/m<sup>3</sup> power input and 20 SLPM air flow rate. Results show the average diameter of 50-100 bubbles at the top and bottom of the representative acrylic tank.

## Volumetric Mass Transfer (k<sub>L</sub>a)

Achieving efficient gas transfer is key to support cell growth, metabolism, and protein production. k<sub>L</sub>a is an important parameter for understanding mass transfer efficiency and the theoretical maximum VCD that the Mobius® iFlex systems can support.

k<sub>L</sub>a was determined via the static gassing out method. Sensors were calibrated to 100% dissolved oxygen (DO) when fully saturated with air. Using nitrogen, the system was purged until the sensor reached <2% DO. Then, air was sparged into the system until the sensor plateaued at the saturated DO level.

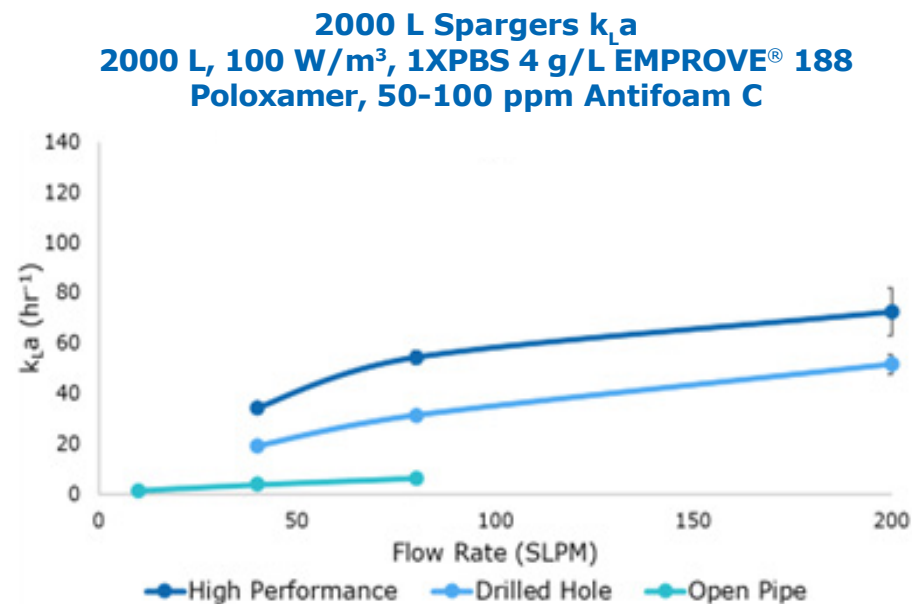
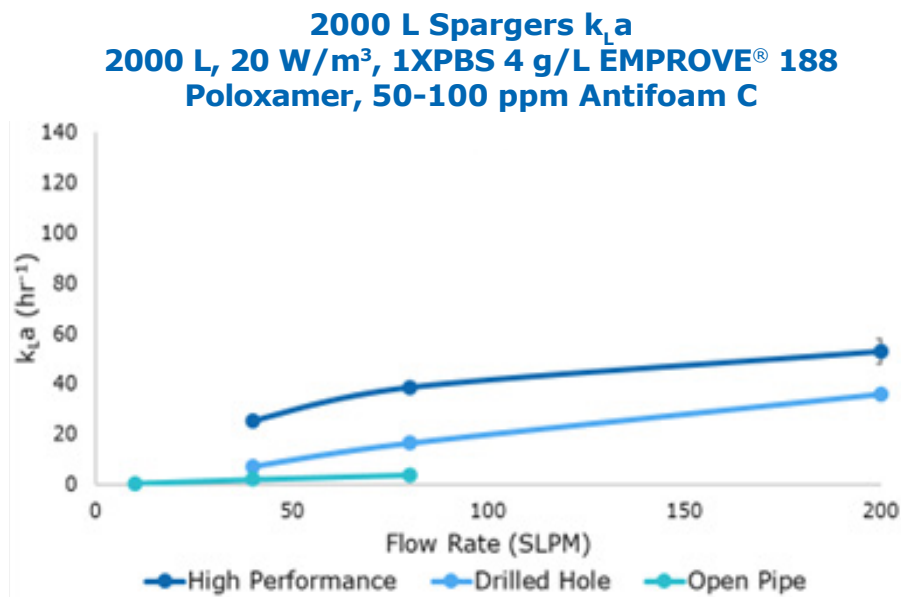
The  $k_La$  value for each trial was calculated from a DO vs time graph. To avoid sensor-related noise in determining the  $k_La$ , the calculation was based on DO concentrations between 10% to 90% of measured air saturation. The  $k_La$  values shown represent the slope of the line created by plotting Eq. 13 versus time ( $t_2 - t_1$ ). The open pipe sparger was tested up to a maximum of 20 SLPM at the 200 L scale and 80 SLPM for the 2000 L scale because it is not expected to be used for oxygenation, therefore has lower recommended operating ranges.



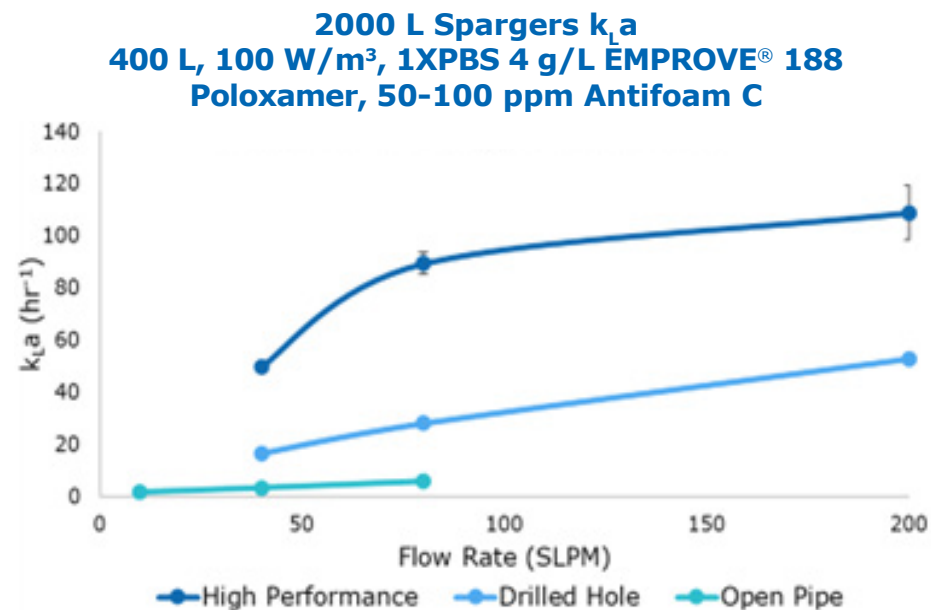
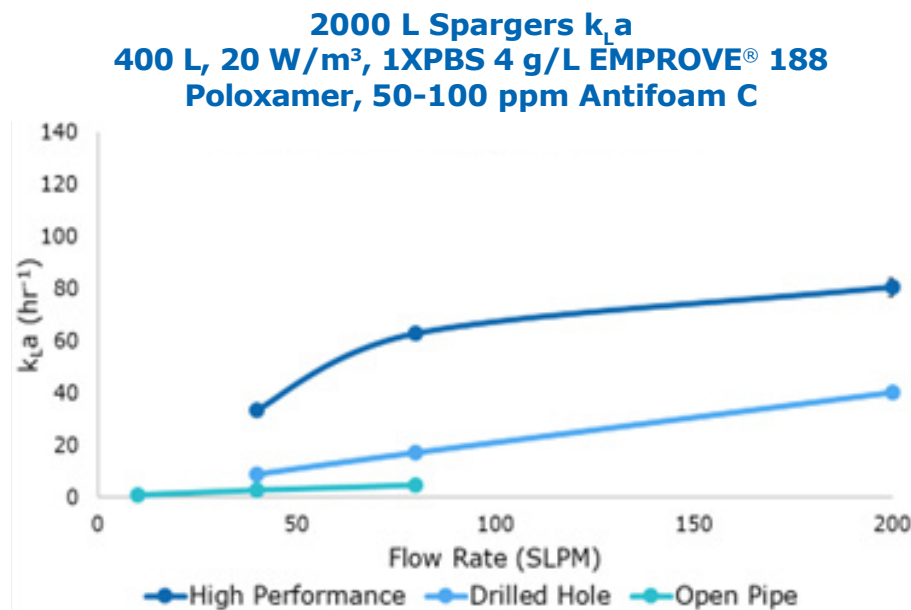
**Figure 5:**  $k_La$  results for high performance, drilled-hole and open pipe spargers with increasing air flowrates at 200 L scale for (G) maximum 200 L and (H) minimum 40 L working volume at 20 and 100 W/m<sup>3</sup>. Results show the average of  $n=3$  runs per condition.



I



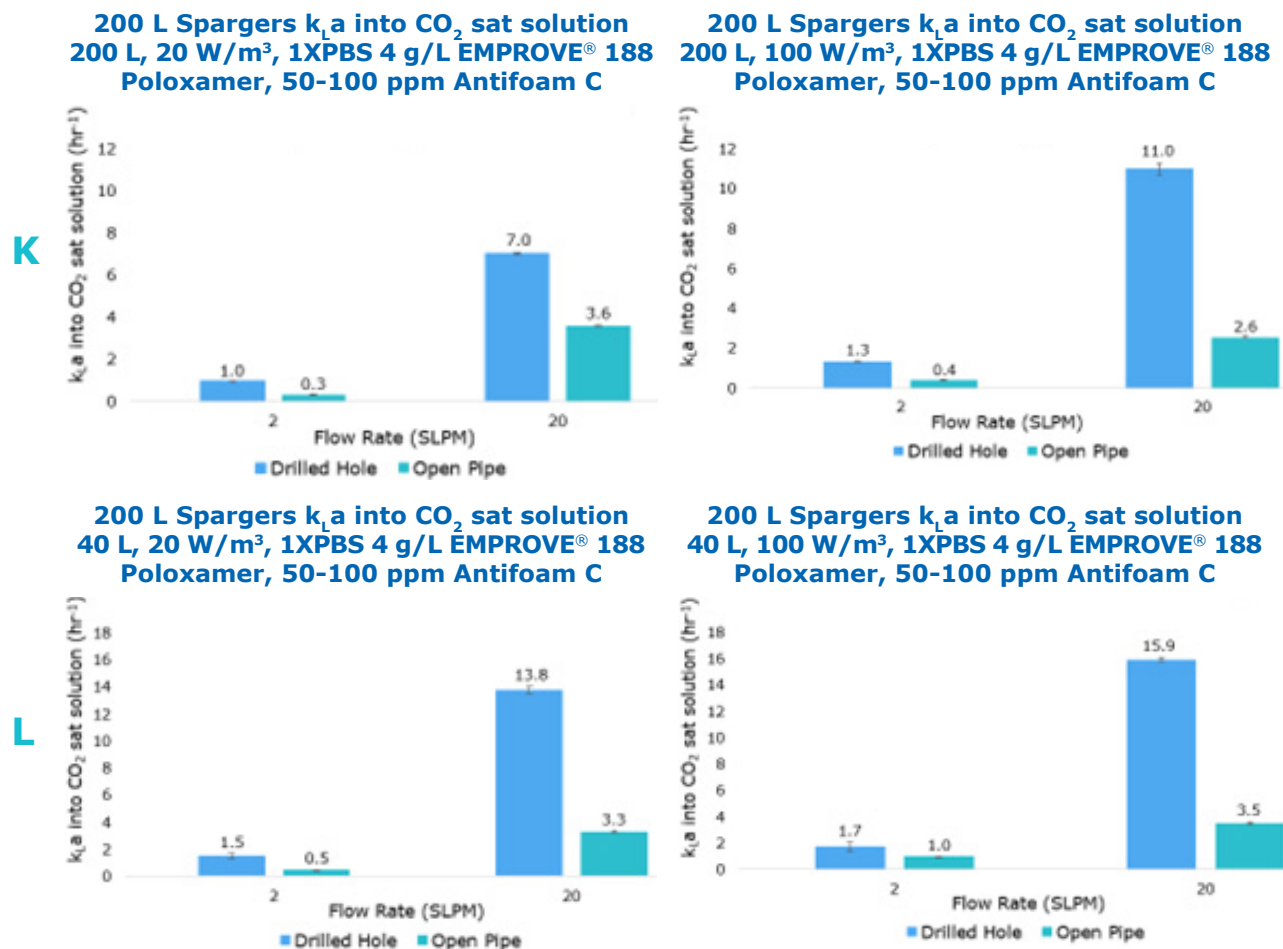
J



**Figure 6:**  $k_L a$  results for high performance, drilled-hole and open pipe spargers with increasing air flow rates at 2000 L scale for (I) maximum 2000 L and (J) minimum 400 L working volume at 20 and 100 W/m<sup>3</sup>. Results show the average of n=3 runs per condition.

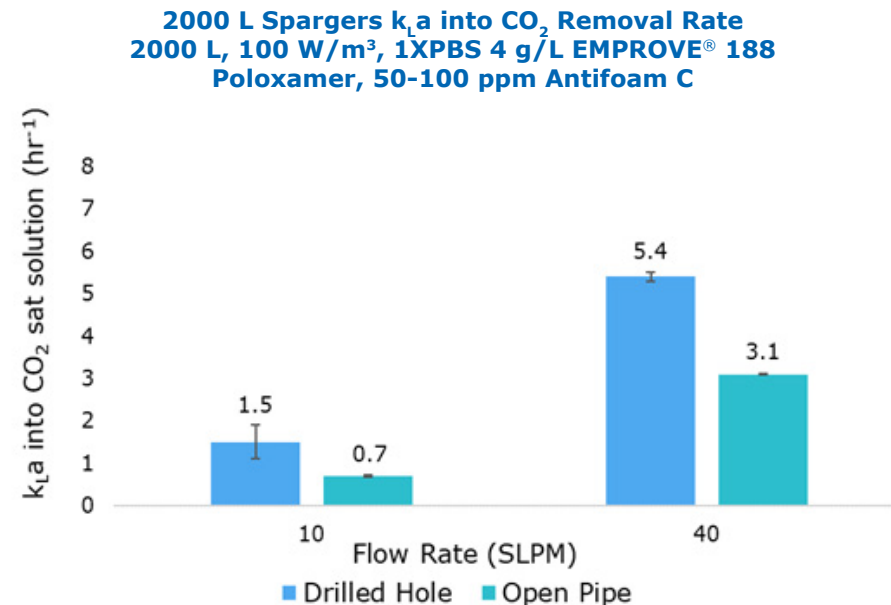
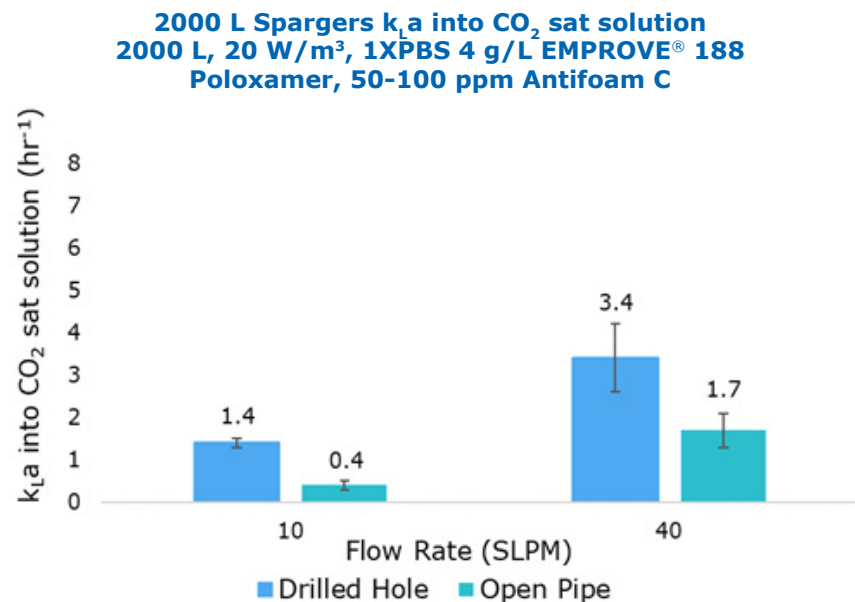
## CO<sub>2</sub> Stripping

Maintaining CO<sub>2</sub> levels within the bioreactor through sparging and stripping is critical to maintain optimal environment for cell growth and protein production throughout the bioprocessing lifecycle. At larger scales, CO<sub>2</sub> can accumulate faster due to the increased bubble residence time, which requires effective stripping capabilities to remove excess CO<sub>2</sub>. To assess the Mobius® iFlex 200 L and 2000 L Bioreactor stripping capabilities, two DO and two CO<sub>2</sub> probes were attached to the system via the sensor ports for data collection. Similar to the k<sub>L</sub>a studies, the static gassing out method was used to determine a k<sub>L</sub>a for air sparged into a CO<sub>2</sub> saturated solution. CO<sub>2</sub> was sparged into the system until saturation reached <10% DO. Air was then introduced via the open pipe or drilled-hole sparger until the solution reached <5% CO<sub>2</sub> and >90% DO. DO data was taken in a range of 10% to 90% and k<sub>L</sub>a was calculated. CO<sub>2</sub> removal rate was also calculated in percent per hour between 10 to 5% CO<sub>2</sub>, a typical working range for cell culture processes. Studies were conducted at maximum and minimum working volume in the Mobius® iFlex 200 L and 2000 L Bioreactor in a solution of 1X PBS with 4 g/L Poloxamer 188 EMPROVE® EXPERT and 50-100 ppm Antifoam C. Only the drilled-hole and open pipe spargers were assessed for CO<sub>2</sub> stripping as these have lower operating flowrate ranges and larger bubble sizes for control of %CO<sub>2</sub>.

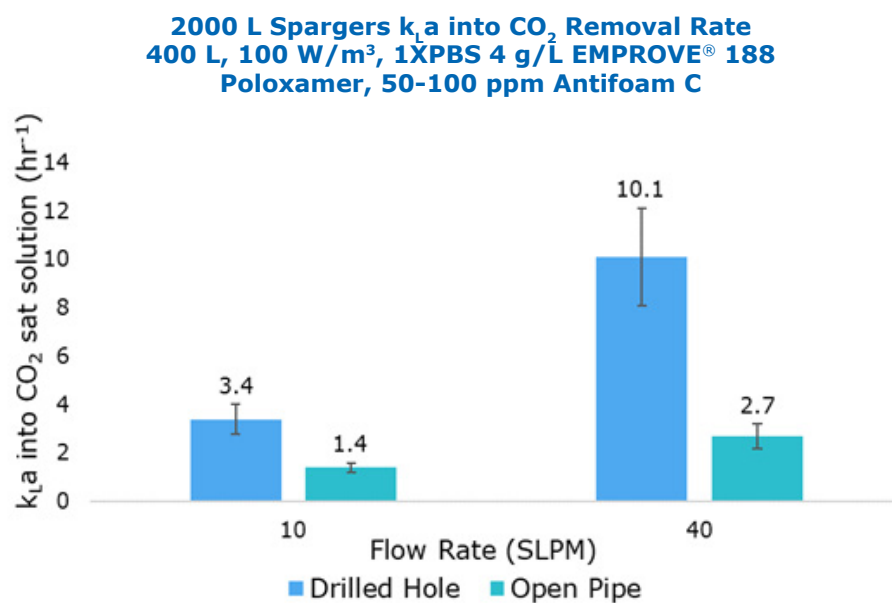
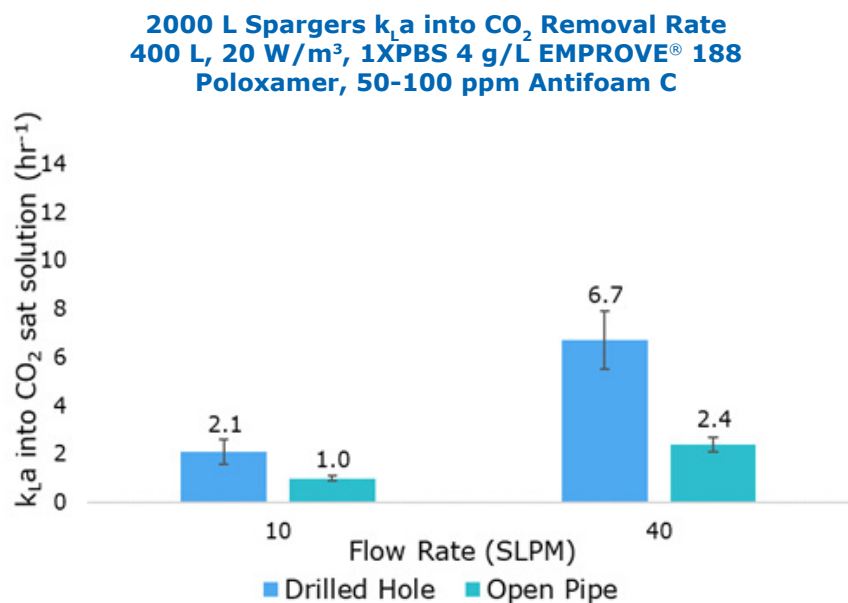


**Figure 7:** k<sub>L</sub>a into CO<sub>2</sub> saturated solution results for drilled-hole and open pipe spargers at 200 L scale, 2 and 20 SLPM air flow rate and (K) maximum 200 L and (L) minimum 40 L volume at 20 and 100 W/m<sup>3</sup> power input. Results show the average of n=2 runs per condition.

M

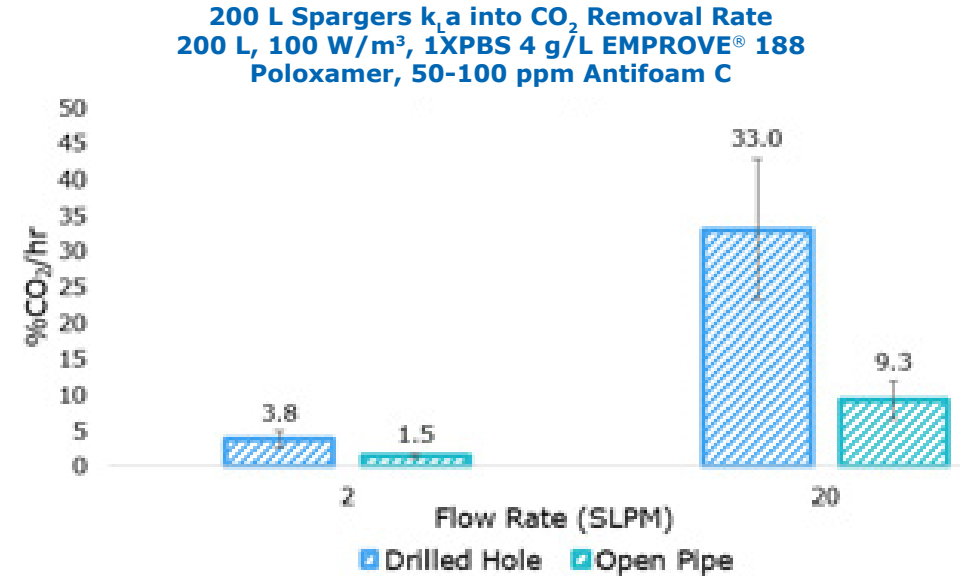
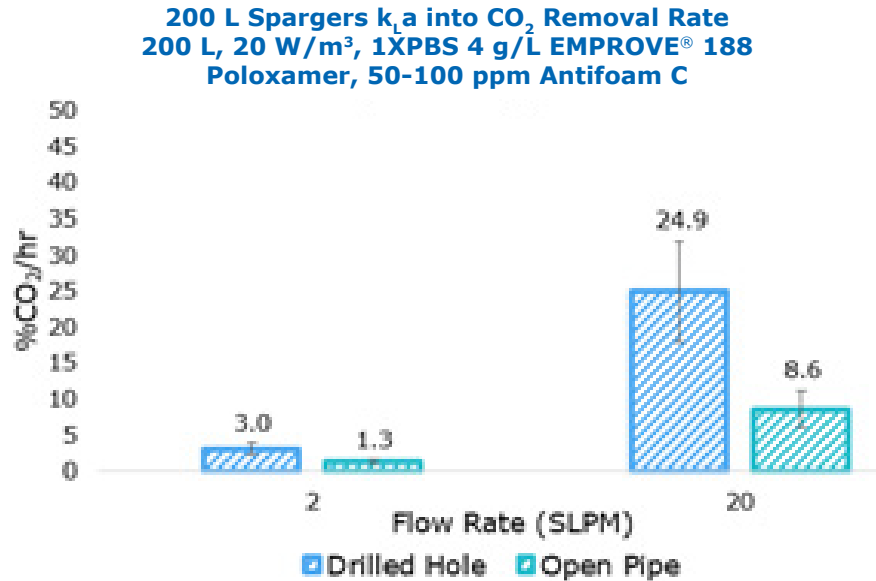


N

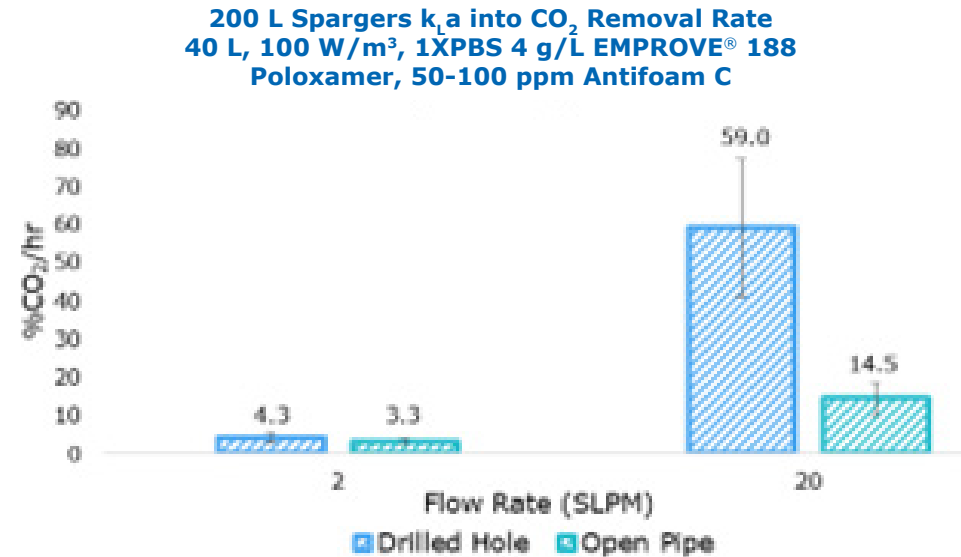
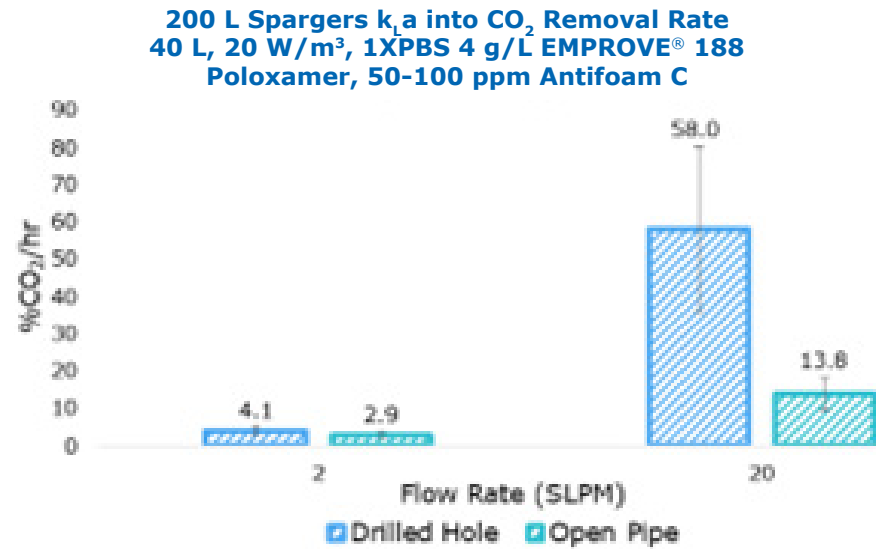


**Figure 8:**  $k_L a$  into  $CO_2$  saturated solution results for drilled-hole and open pipe spargers at 2000 L scale, 10 and 40 SLPM air flow rate and (M) maximum 2000 L and (N) minimum 400 L volume at 20 and 100 W/m<sup>3</sup> power input. Results show the average of  $n=2$  runs per condition.

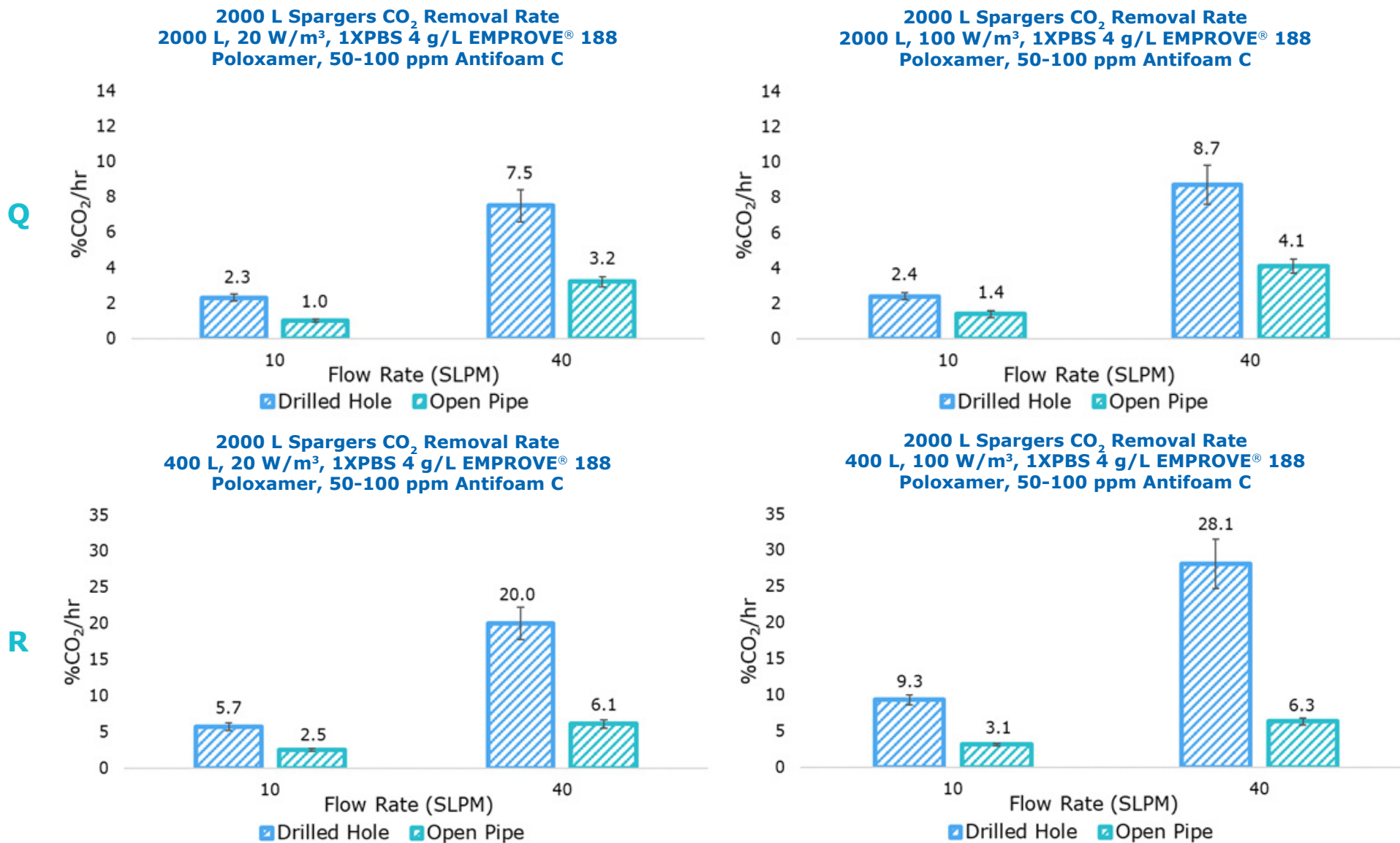
O



P



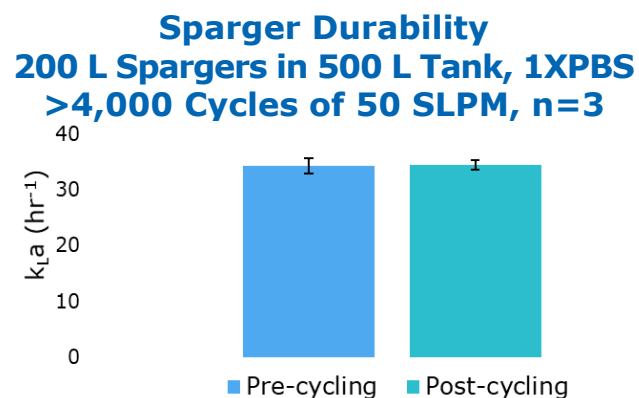
**Figure 9:**  $CO_2$  removal rate results for drilled-hole and open pipe spargers at 200 L scale, 2 and 20 SLPM air flow rate and (O) maximum 200 L and (P) minimum 40 L volume at 20 and 100 W/m<sup>3</sup> power input. Results show the average of n=2 runs per condition.



**Figure 10:** CO<sub>2</sub> removal rate results for drilled-hole and open pipe spargers at 2000 L scale, 10 and 40 SLPM air flow rate and (Q) maximum 2000 L and (R) minimum 400 L volume at 20 and 100 W/m<sup>3</sup> power input. Results show the average of n=2 runs per condition.

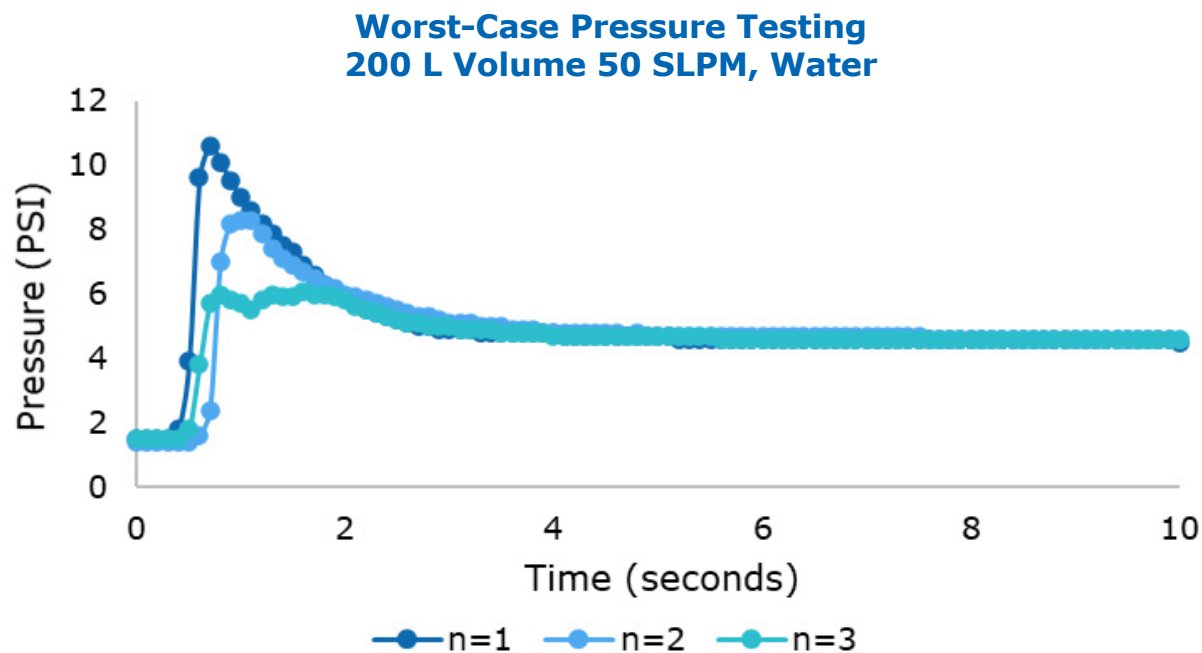
## Stress Testing

The high performance sparger was subjected to pulse testing within a 500 L acrylic tank system to simulate worst case scenario of pressure cycling that could occur within a poorly controlled intensified run. Flow rates of 50 SLPM air were run for 1-minute intervals with a 30 second pause in between, totaling > 4,000 cycles to represent the equivalent of 100 on/off cycles/day for 40 days.  $k_La$  was assessed before and after pressure cycling using the static gassing out method to determine material and design durability. Results showed no significant change in  $k_La$  pre- and post-pressure cycling.



**Figure 11:** Worst-case scenario pressure cycling test results for the 200 L high performance sparger in the 500 L acrylic tank.  $k_La$  was measured n=3 times pre and post pressure cycling.

Worst-case scenario pressure tests were also conducted on the high performance sparger at the 200 L scale to simulate the sparger filling with liquid, then air being introduced at the maximum flow rate through the sparger. This was simulated by adding a tee fitting near the sparger inlet port to monitor pressure. Pressure was monitored and collected using a PendoTECH® pressure sensor. Pressure in the sparger was zeroed with no flow to the sparger in open air. The system was then filled with water, and the tubing connected to the sparger was also filled with water by electronic pipette. Air was then flowed through the filled sparger at 50 SLPM with no mass flow controller ramp rate to simulate worst-case scenario. The highest pressure observed after n=3 trials was 10.6 psi, confirming that the sparger holder caps and sparger designs can sufficiently withstand the highest expected pressure. Additional testing confirmed the sparger holder cap can withstand 2x the maximum expected pressure (results not shown).



**Figure 12:** Worst-case scenario liquid fill pressure test results for the 200 L High Performance sparger. Pressure profiles were monitored over time for n=3 runs.

## Duration Study

For the duration study described previously (refer to [Duration Study](#)), drilled hole and high performance spargers were operated at nominal flowrates of 12 SLPM for the 200 L scale and 50 SLPM for the 2000 L scale of air continuously for 40 days. The spargers operated effectively throughout the duration of the test.

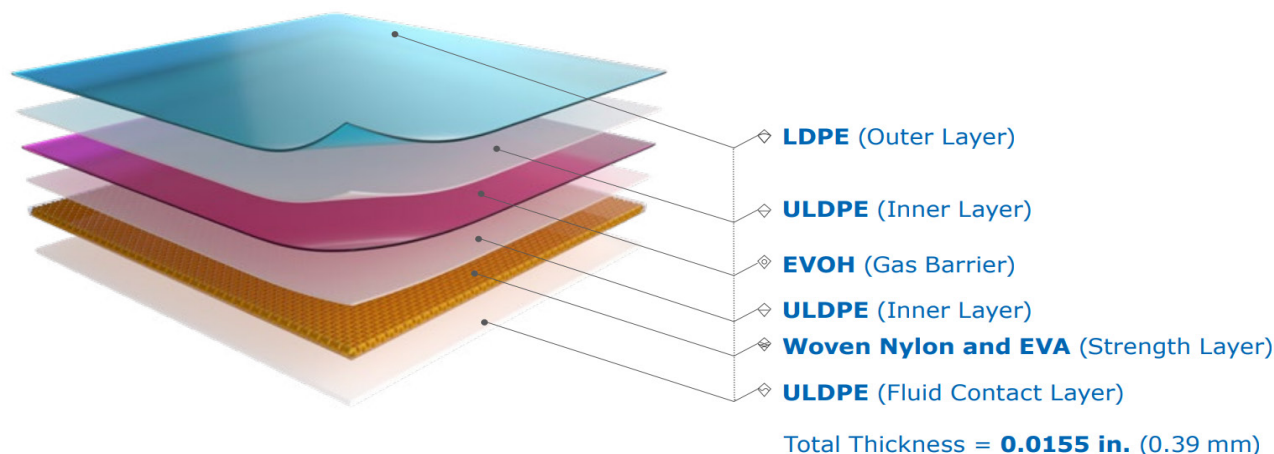
### Summary

- Sparger designs were optimized for shear and scalability considerations. Novel scalability strategy using maximum flow rate and constant gas velocity allows for predictable performance.
- Spargers support high cell densities through oxygenation and optimized CO<sub>2</sub> stripping as demonstrated by in-depth characterization of  $k_L a$  and CO<sub>2</sub> stripping.
- Three spargers are included in each bag assembly to provide a wide range of gassing strategy options to meet the oxygen transfer rate, CO<sub>2</sub> stripping, and the bubble size and shear considerations specific to the process needs.
- Designs are robust through drilled-hole material choices and characterized through worst-case testing to reduce risk of use.

## Component Performance

### Ultimus® Film

The integrity of the film used in single-use systems is critical to maintaining product quality and sterility, and reliable manufacturing operations. Mobius® iFlex Bioreactor bags are constructed of Ultimus® film. Ultimus® film was developed as a single-use solution to meet the challenges of large volume processing, and has superior abrasion and tear resistance, puncture strength and durability.<sup>11</sup> Specifically, the strength layer is composed of a woven nylon that exponentially improves the durability and strength of the film. In addition, Ultimus® film is Irgafos® 168 free to support healthy cell growth performance. When incubated with media, Ultimus® bags demonstrated performance within normal culture variations for growth, viability, protein production and product quality when compared to glass bottles.<sup>12</sup>



**Diagram 8:** *Ultimus® Film Structure*

### Duration Study

Durability of the Ultimus® film assembly was also investigated during the duration study (refer to [Duration Study](#)) and showed no signs of significant damage after the 40 day continuous duration.

## Probe Performance

**Note** Single-use probe variations were gamma sterilized before testing.

### Dissolved Oxygen (DO) Probes

To monitor dissolved oxygen during cell culture, two single-use Hamilton VisiFerm Arc DO probes come pre-installed within the single-use Ultimus® bag. A multi-use Hamilton DO probes kit is also available. To characterize the performance of the DO probes, probe drift and linearity were tested against a calibrated, regularly checked reference probe (Endress+Hauser, Digital optical oxygen sensor Memosens COS81E). Probe drift was measured by installing three probes (reference, single-use, multi-use) in a 3 L bioreactor filled with 1X PBS for 35 days with mixing. The bioreactor was open to the room to maintain an air-saturated solution. Over the duration of the study, there was no drift between the single-use and multi-use probes. Compared to the reference probe, which was calibrated in air prior to the endurance test, there was a drift of 2.1%. To assess linearity, a 3 L bioreactor filled with 1X PBS at room temperature and 250 RPM agitation speed was connected to two gas bottles (air and N<sub>2</sub>) via a feed line.

The single-use or multi-use probe was installed in the bioreactor and air was sparged in until stability reached 100% saturation. The fully saturated value was used as the process calibration point. N<sub>2</sub> was then sparged until DO reached <15%. Linearity was confirmed by graphing the reference probe DO values against the Hamilton probe values, resulting in a high R<sup>2</sup> correlation of > 0.999 for both the single-use and multi-use probe options.

### CO<sub>2</sub> Probes

To monitor %CO<sub>2</sub> in the bioreactor, a multi-use Hamilton CO<sub>2</sub>NTROL RS485 120 probe is available. The probe accuracy reported from the supplier is ±5% of the process value (<100 mbar).

### pH Probes

To monitor pH during cell culture, two Hamilton OneFerm single-use probes come pre-installed in the single-use Ultimus® bag. A multi-use Hamilton pH probes kit is also available. The stability of the pH measurement was tested by continuous operation in mock cell culture media (150 mM PBS with pH of 7.4 and conductivity of 17 mS/cm) for 25 days without calibration during the test. A Hamilton OneFerm Single-use probe and Hamilton Polilyte Multi-use probe were set-up within a test bench loop. The single-use and multi-use probes had variations of 0.07 and 0.01 pH units respectively at the end of the 25-day run. Six single-use probes were also tested for calibration accuracy by conducting a 1-point calibration at pH 7. Standard buffer solutions between pH 6-8 were used for testing (n=4). Of the 24 trials, 23 of them passed the ±0.1 pH unit acceptance criteria.



### Viable Cell Density (VCD) Probes

For VCD probe options, the Mobius® iFlex Bioreactor is compatible with both single-use or multi-use Hamilton Incyte permittivity probes. The performance of both probes was compared against each other for permittivity and conductivity accuracy. To measure conductivity, probes were submerged in a 25 or 50 g/L NaCl solution. A calibrated, regularly checked reference probe (Endress+Hauser) was also tested to obtain a theoretical baseline value. Results were reported in percent relative error of baseline. To test permittivity, cell culture of a suspended CHO clone (ZN-GS, provided by MilliporeSigma) producing a monoclonal antibody was inoculated at a starting density of  $5 \times 10^5$  viable cells/mL. The study was run in a 3.5 L bioreactor (BiFlo® 320, Eppendorf) with a 3 L starting volume. The culture medium used was EX-CELL® Advanced HD Perfusion Medium with temperature control at 37°C, dissolved oxygen control at 40% by enriched O<sub>2</sub> flow and pH control at 7 ( $\pm 0.05$ ) by base NaOH and CO<sub>2</sub> additions. From day 3 onward, Cellvento® 4Feed (SAFC) was added to the bioreactor regularly. For superior titer performance, 150 mL EX-CELL® Advanced™ CHO Feed 1 7.5%v/v (SAFC) was used in conjunction with the prior feed. Glucose (2 g/L) was also supplemented as needed along the 14-days of culture. A recirculation loop was connected to the bioreactor to test the multi-use and single-use probes.

The loop had a recirculation time of 36 seconds with an equivalent flowrate of 340 mL/min. Permittivity curves were measured at 300 kHz throughout the culture duration. The average relative error between the multi-use and single-use probes was 1.72% throughout the process, indicating that both probe formats measure permittivity with the same accuracy.

**Table 6**

Results of the relative percent error in conductivity values compared to baseline for multi-use and single-use VCD probes:

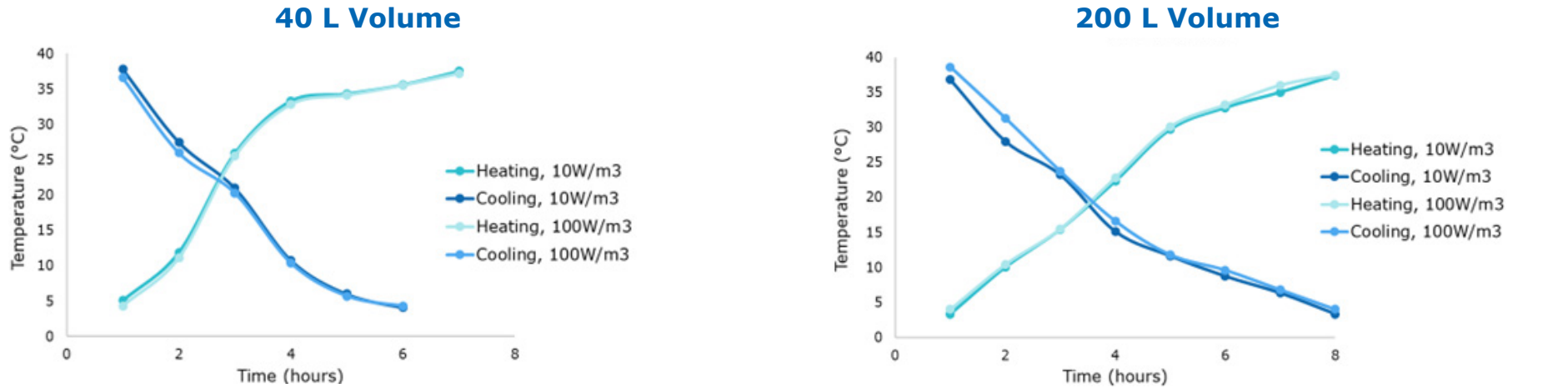
| NaCl Concentration (g/L) | Baseline Conductivity (mS/cm) | Relative Error (%) |            |
|--------------------------|-------------------------------|--------------------|------------|
|                          |                               | Multi-use          | Single-use |
| 25                       | 40.1 $\pm$ 1.0                | 0.2                | 0.9        |
| 50                       | 78.6 $\pm$ 3.1                | 6.7                | 4.8        |

### Tubing Duration

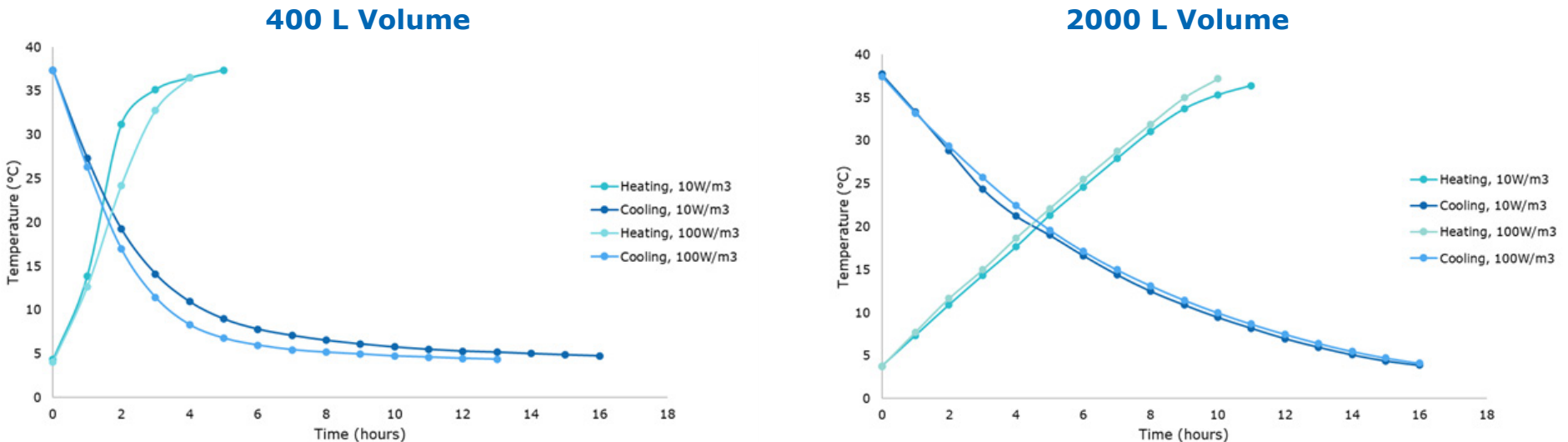
For cell culture applications, peristaltic pumps are used for liquid additions or removals. To ensure durability of the tubing under operating conditions, PharMed and Bioprene (for perfusion) tubing were tested continuously for 30 days at the peristaltic pumps' maximum operating flowrate. The tubing showed no signs of leaking, damage to the internal surfaces or generated particles during this time. The output flowrate was also within  $\pm 10\%$  of the target at the start and end of the test indicating consistent performance for 30 days of pump exposure.

## Heat Transfer

Bioreactor operation relies on precise temperature control of the cell culture. Having efficient temperature control is vital to prepare the cell inoculation step. To ensure efficient heating and cooling rates, the 200 L and 2000 L Mobius® iFlex bioreactor jacketed vessels were connected to a temperature control unit (VC2000, Lauda for the 200 L scale and VC10000, Lauda for the 2000 L scale) to heat and cool the bioreactor from 4°C to 37°C and 37°C to 4°C. Liquid temperature was monitored with two temperature probes in the bioreactor. Further optimization of heating/cooling speeds is possible through PID tuning.



**Figure 13:** Heating and cooling profiles at 200 L scale for minimum 40 L and full 200 L volume at 10 and 100 W/m<sup>3</sup>



**Figure 14:** Heating and cooling profiles at 2000 L scale for minimum 400 L and full 2000 L volume at 10 and 100 W/m<sup>3</sup>

**Table 7**

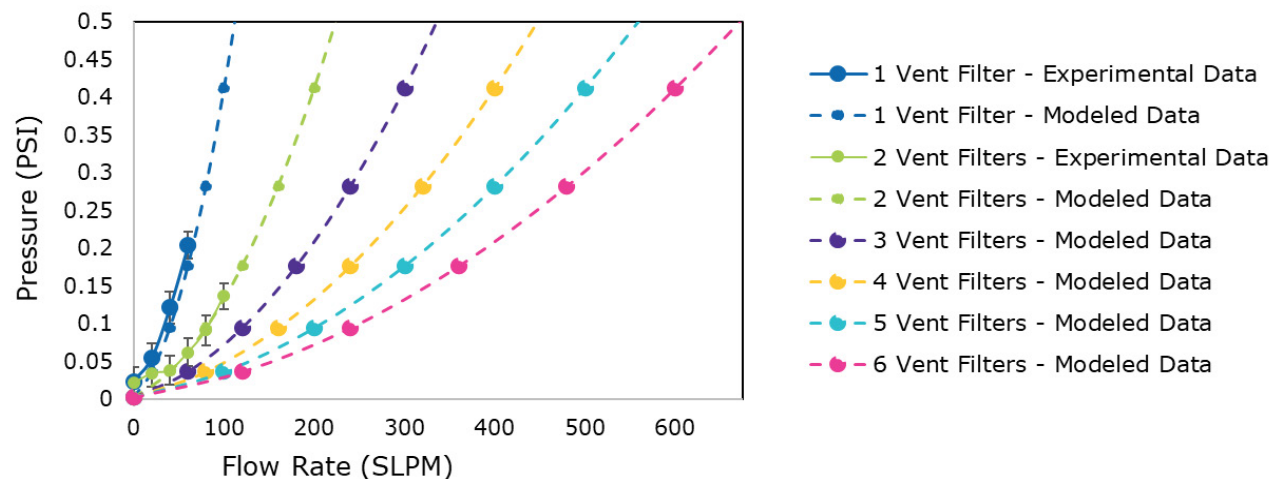
Temperature control unit (TCU) recommendations per scale:

| Scale (L) | TCU Recommendation |
|-----------|--------------------|
| 50        | Lauda RE30S        |
| 200       | Lauda VC 2000      |
| 500       | Lauda VC 5000      |
| 1000      | Lauda VC 10000     |
| 2000      | Lauda VC 10000     |

## Vent Filters

Bioreactor operation requires sterile vent filters to allow venting at maximum exhaust flow that can support oxygenation demands. Exhaust vent filters should generate minimum back pressure within the single-use bioreactor bag at the maximum flow rate to ensure bag integrity. Therefore, vent filter design and quantity must be considered for effective operation. To test the design requirements, vent filters (Aervent® Opticap XL5) were heated to 40°C in the 200 L Mobius® iFlex bioreactor. The single-use bag assembly was filled with 200 L of water at 37°C and agitation was run at 50 W/m<sup>3</sup> power input. Gas was sparged into the bioreactor via the drilled-hole and open pipe spargers for total flow rates up to 100 SLPM. Pressure was monitored with a single-use PendoTECH® pressure sensor and trigger shut-off of gas supply if bag pressure exceeded 0.5 psi (34.5 mbar). Curves of flowrate vs. pressure showed that even one vent filter was sufficient to support the maximum flow rates delivered by the mass flow controllers at 200 L scale, which is in alignment with the single-use bag design that has a total of 3 connectors for vent filter assemblies. Vent filter performance was also estimated up to 2000 L scale through Eq. 14 using experimental data of one vent filter. The model equation showed agreement with the estimation for two vent filters.

**Number of Vent Filters Comparison, Scalability 40°C Liquid Temperature, 40°C Vent Heaters, Full Volume**



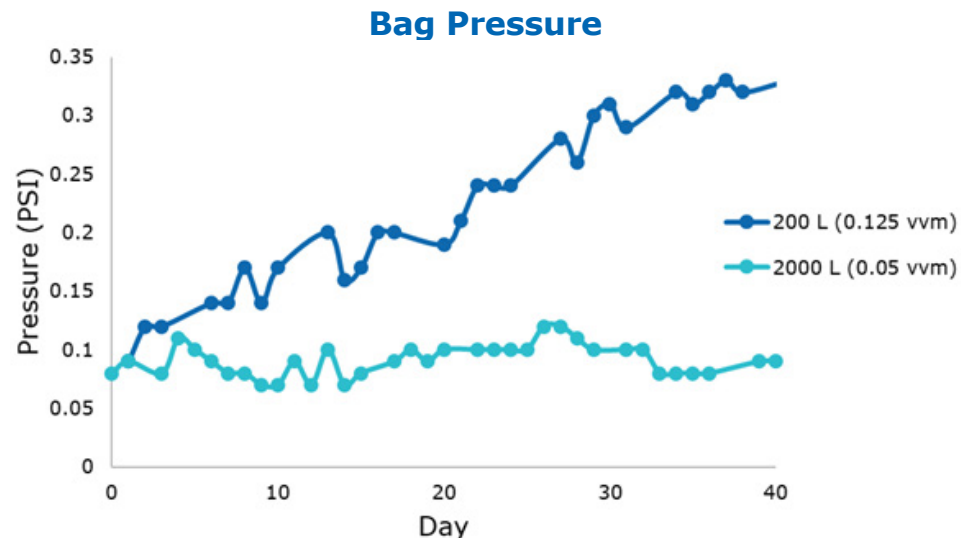
**Figure 15:** Modeled pressures for 1-6 vent heaters for various gas flowrates. Experimental data is shown for the 200 L system with 1 or 2 vent filters overlaid with the solid line. Dotted lines represent modeled data.

**Table 8**

Number of vent filters covering operation up to 1.5X the maximum sparger flowrate for 50 L to 2000 L scales:

| Scale (L) | 1.5x Maximum Sparger Flow Rate- Expected Maximum (SLPM) | Number of Vent Filters (design includes 1 back-up) |
|-----------|---|--|
| 50        | 30  | 2  |
| 200       | 75  | 3  |
| 500       | 150   | 4  |
| 1000      | 225   | 5  |
| 2000      | 300   | 6  |

Vent filter performance was also assessed during the 40-day continuous duration study at nominal conditions by monitoring bag pressure. Two vent filters were connected to the 200 L Mobius® iFlex single-use bag assembly and five vent filters were connected to the 2000 L Mobius® iFlex single-use bag assembly and heated to 50°C. The bag was filled with water to maximum volume and heated to 37°C. The high performance sparger and drilled-hole sparger were run continuously at 12 SLPM (0.125 vvm) air each for the 200 L scale and 50 SLPM (0.05 vvm) air each for the 2000 L scale. Bag pressure was monitored via the single-use PendoTECH® sensor with a safety shut-off of 0.5 PSI for the 200 L scale and 0.4 PSI for the 2000 L scale. Over the 40-day duration, bag pressure did not exceed 0.35 PSI for the 200 L scale and did not exceed 0.15 PSI for the 2000 L scale, staying within safety limits and demonstrating adequate exhaust flow for two vent filters in the Mobius® iFlex 200 L Bioreactor and five vent filters in the Mobius® iFlex 2000 L Bioreactor at nominal operation.



**Figure 16:** Bag pressure over time for a 40-day duration test at nominal conditions for the 200 L scale with two vent filters shown at 0.125 vvm and the 2000 L scale with five vent filters shown at 0.05 vvm.

## Check Valves

Check valves are used in the bioreactor single-use assembly to prevent backflow of fluid in the sparger lines. Two sizes of check valves, ¼-inch and ½-inch, are incorporated in the Mobius® iFlex single-use bags through barbed fittings on the sparger gas lines. The durability of both types were assessed by flowing air through gamma-irradiated samples into a partially filled 500 L representative acrylic tank for a back pressure of about 2.5 psi. Three ¼-inch check valves were tested for 24 hours at 100 SLPM, cycling for 30 seconds on and 90 seconds off (>700 cycles total). Nine ½-inch check valves were tested for 7 days at 200 SLPM, cycling for 30 seconds on and 90 seconds off (>5000 cycles total). Leaks and minimum pressure/flow checks were performed before and after durability testing. All tested valves passed without failure, showing no change in performance over the expected use of the valve. Pressure drop and opening pressure remained consistent throughout testing, and no gas or water leaks were detected.

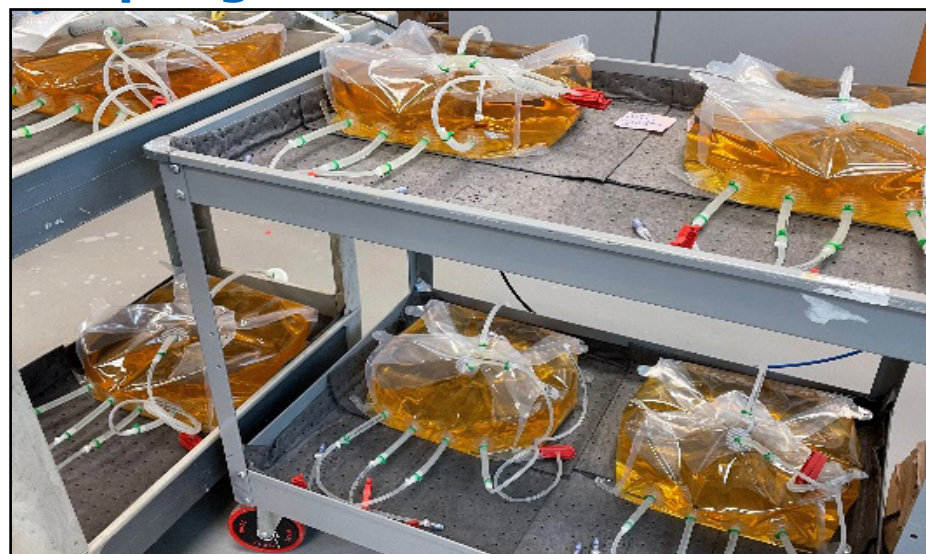


**Diagram 9:** ¼-inch Nordson Medical check valve for the 200 L scale



**Diagram 10:** ½-inch Smart Products check valve for the 2000 L scale

## Sampling



**Diagram 11:** Sampling set-up

A durability study was conducted on the sampling line to ensure there were no areas of concern with sampling for durations up to 40 days. A total of 6 custom bags made with Pureflex™ film were assembled with 3 representative sampling lines each. The bags were all filled with approximately 10 L of sterile BD Bacto Tryptic Soy Broth. Each of the lines were sampled 40 times over the 40-day period. Four of the 6 bags were sampled using Method 1 (refer to Table 9), which involved cleaning, clamping, and purging the line prior to taking a sample. The other 2 bags were sampled using Method 2 (refer to Table 9) with no cleaning or clamping of the line prior to taking the sample. Both sampling methods maintained media sterility over the 40-day testing period. However, it is recommended to follow method 1 for sampling procedures to ensure sterile conditions.

**Table 9**

Sampling methods:

| Method 1   | Method 2                             |
|--|--------------------------------------|
| 1. Spray gloved hands with alcohol.                    | 1. Attach syringe to the line.       |
| 2. Spray and clean the outside of the sampling port.   | 2. Purge a full syringe.             |
| 3. Ensure the line is clamped. If not, clamp the line. | 3. Slowly remove syringe.            |
| 4. Attach syringe to the line.                         | 4. Attach a new syringe to the line. |
| 5. Open the clamp and purge a full syringe.            | 5. Draw the sample.                  |
| 6. Close the clamp and slowly remove syringe.          | 6. Slowly remove syringe.            |
| 7. Spray and wipe sampling port again.                 |                                      |
| 8. Attach a new syringe to the line.                   |                                      |
| 9. Open the clamp and slowly draw the sample.          |                                      |
| 10. Close the clamp and slowly remove syringe.         |                                      |
| 11. Clean outside of the sampling port.                |                                      |

**Summary**

- Durability and duration testing of the system components showed passing performance results at maximum or worst-case scenario operating conditions.
- Vent filter performance is robust as demonstrated by a 40-day continuous duration.

## Conclusion

Intensified bioprocesses require an expanded system design space to support demands of higher cell densities while being flexible and scalable across sizes. Physical properties such as mixing time, volumetric mass transfer capabilities, and power input define the process design space wherein systems can accurately and effectively monitor and control critical process parameters. Component durability and duration testing confirms that designs are robust and capable of supporting targets over the duration of a process. Scalability studies in representative systems provide a guide for process scale up across the range of system sizes from 50 L to 2000 L. The collective results of these studies confirm that the Mobius® iFlex Bioreactors accommodate a wide range of performance capabilities for supporting various biomanufacturing processes.

# Appendix

## Equations

| Equation |  |   | Variables  |
|----------|--|---|--|
| 1        | Power Density                              | $P_d = P/V$   | P = power<br>V = volume  |
| 2        | Angular Velocity                           | $\omega = \text{RPM}_{\text{avg}} * (1 \text{ min}) / (60 \text{ sec})$ |  |
| 3        | Power                                      | $P = \tau * \omega$   | $\tau$ = torque<br>$\omega$ = angular velocity   |
| 4        | Power Number                               | $N_p = P / (\rho d^5 \omega^3)$   | $\rho$ = density of mixing fluid<br>d = impeller diameter  |
| 5        | Reynold's Number                           | $Re = (\omega d^2 \rho) / \mu$  | $\mu$ = viscosity of mixing fluid  |
| 6        | Tip Speed                                  | $v_{\text{tip}} = \omega * d * \pi$                                     | d = impeller diameter  |
| 7        | Tip Shear Rate                             | $\gamma_{\text{tip}} = 2\pi DN/H$                                       | D = impeller diameter<br>H = impeller height<br>N = rotational velocity  |
| 8        | Cup Shear Rate                             | $\gamma_{\text{cup}} = (2\pi R_i N) / (R_o - R_i)$                      | $R_i$ = inner cup radius<br>$R_o$ = outer cup radius   |
| 9        | Gas Entrance Velocity                      | (Flow Rate) / (Open Area (Number of Holes * Hole Area))                 |  |
| 10       | Bubble Diameter                            | $D = \sqrt{(\text{min } feret * \text{max } feret)}$                    | min <i>feret</i> = minimum bubble diameter<br>max <i>feret</i> = maximum bubble diameter   |
| 11       | Mass Transfer Mass Balance at steady state | $OTR = OUR = VCD * q_{O_2} = k_L a (C^* - C)$                           | OTR = oxygen transfer rate<br>OUR = oxygen uptake rate<br>VCD = viable cell density<br>$q_{O_2}$ = cell specific oxygen uptake rate<br>$k_L a$ = volumetric mass transfer rate |
| 12       | Mass transfer coefficient ( $k_L a$ )      | $k_L a = OTR / (C^* - C)$   | $C^*$ = saturation concentration<br>C = oxygen concentration<br>$C_1$ = initial gas concentration  |
| 13       | Mass transfer coefficient ( $k_L a$ )      | $\ln((C^* - C_1) / (C^* - C)) = k_L a * (t - t_1)$                      | t = time<br>$t_1$ = initial time   |
| 14       | Number of vent filters based on flow rate  | $(\text{Flow rate})_n = (\text{Flow rate})_1 * n$                       | n = number of vent filters   |

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