

Performance Guide

Mobius® CellReady 50 L and 200 L Single-use Bioreactor Systems



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The Mobius® CellReady 50 L and 200 L single-use bioreactors

The Mobius® CellReady 50 L and 200 L bioreactors are single-use, stirred tank bioreactors designed for pilot and clinical scale mammalian cell culture applications. Characterization of mixing time, volumetric mass transfer capabilities, temperature mapping and power input was completed to define the process design space wherein accurate and effective monitoring and control can occur within the Mobius® CellReady 50 L and 200 L bioreactors. In addition to physical characterization of the processing design space, studies were executed to demonstrate the cell culture capabilities of the Mobius® CellReady bioreactors for 10 L through 200 L working volume capacity.

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Mobius®

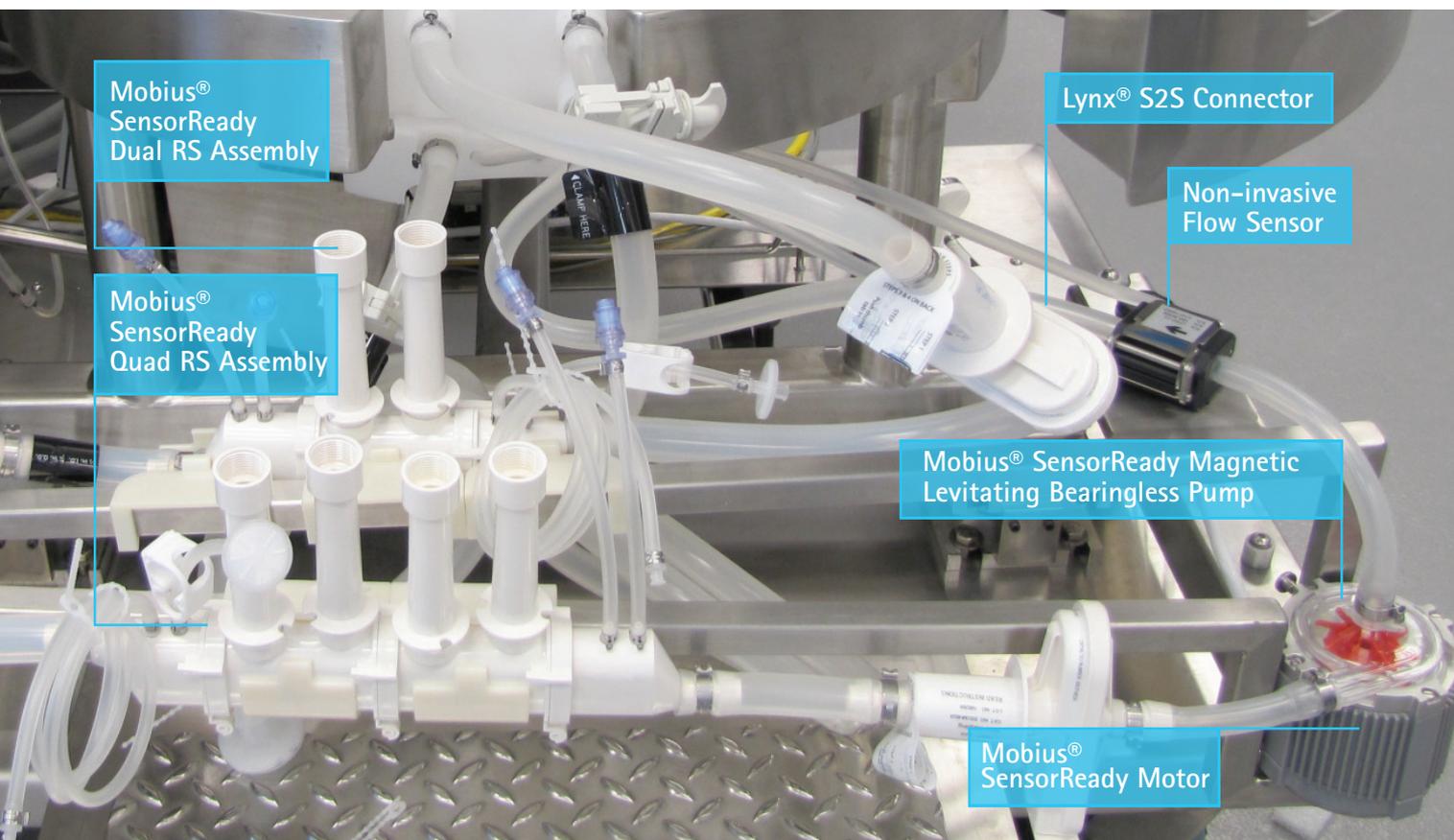
SensorReady technology



Innovative and flexible process monitoring

The Mobius® CellReady single-use bioreactors are uniquely designed with the novel Mobius® SensorReady technology, an external loop used to monitor and control the bioreactor. This technology provides flexible and configurable process monitoring capabilities at the point of use, reducing the need to customize bioreactor process containers and enabling easy integration of new sensor technologies.

The Mobius® SensorReady assembly consists of a continuous flow loop connected to the bioreactor process container using the validated sterile Lynx® S2S connector. A fixed flow rate of 3 LPM is consistently maintained in the loop using a low shear, bearingless centrifugal pump and a non-invasive flow meter to signal any disruptions in the continuous flow. Two options are available for sampling; a closed loop sampling option fitted with weldable C-Flex® tubing and two ports fitted with needle-free swabable valves.



Mixing

The Mobius® CellReady 50 L and 200 L bioreactor process containers are designed to provide a well-mixed cell culture environment across a wide range of working volumes by combining an up-pumping bottom-mounted pitched blade impeller, offset from center 15°, with a single baffle. Mixing time in both the Mobius® CellReady 50 L and 200 L bioreactor process containers was evaluated by observing conductivity probe response curves measured at four locations within the system. T_{95} mixing time at the four probe locations was determined as the time when conductivity profiles had reached 95% of the final value for each of the three agitation rates tested. The Mobius® SensorReady assembly for this experiment consisted of two Quad RS modules in series, representing the maximum configuration of the system. One conductivity probe was located in the 8th (i.e., the last) probe port in the Mobius® SensorReady assembly, and the other three probes were placed in the top, middle and bottom of the bulk bioreactor process container. Each bar shown in Figure 1A represents mixing trials in which a volume of 750 mL of aqueous salt solution (NaCl) was introduced to the 50 L bulk volume as a bolus feed at the liquid surface. Each bar shown in Figure 1B represents mixing trials in which a volume of 3 L of aqueous salt solution (NaCl) was introduced to the 200 L bulk volume as a bolus feed at the liquid surface. The bulk average value (Bulk Ave) is derived from averaging the three probes placed in the bioreactor process container (Top, Middle and Bottom), and the system average (System Ave) indicates the average of all four conductivity probe T_{95} mixing times.

- At each agitation rate tested, the fastest mixing time recorded was in the bottom of the bioreactor process container indicating that the impeller-baffle interaction creates a fluid pattern that draws material from the top of the bioreactor process container downward. This was confirmed visually with dye studies (data not shown).
- At the middle and high end of the agitation range, the greatest difference in mixing time detected at the four probe locations was between the top and bottom of the bioreactor process container, which differed by approximately 10 -12 seconds, indicating good homogeneity within both the 50 L and 200 L systems.
- At the lowest agitation rate tested, the greatest difference in mixing time detected at the four probe locations was between the top and bottom of the tank, which differed by approximately 16 seconds in the 200 L and 12 seconds in the 50 L bioreactor process container, indicating that mixing homogeneity can be improved with increased agitation rates.

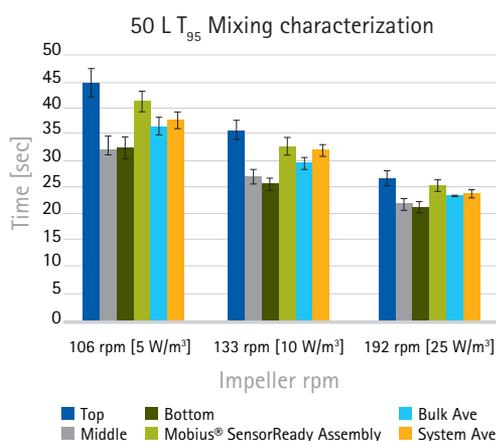


Figure 1A.

Mixing in 50 L Mobius® CellReady bioreactor process container (50 L volume). Each bar is an average (n=15). The error bars indicate 1 standard error from the 15 readings of T_{95} .

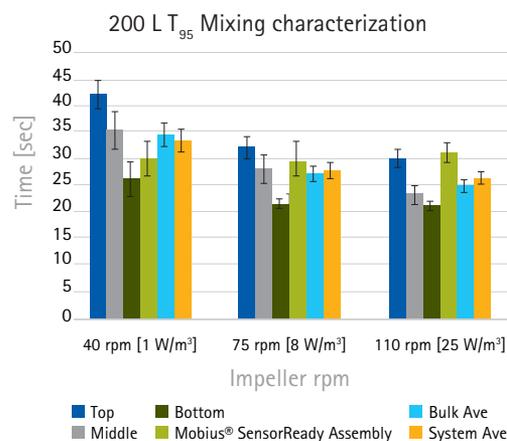
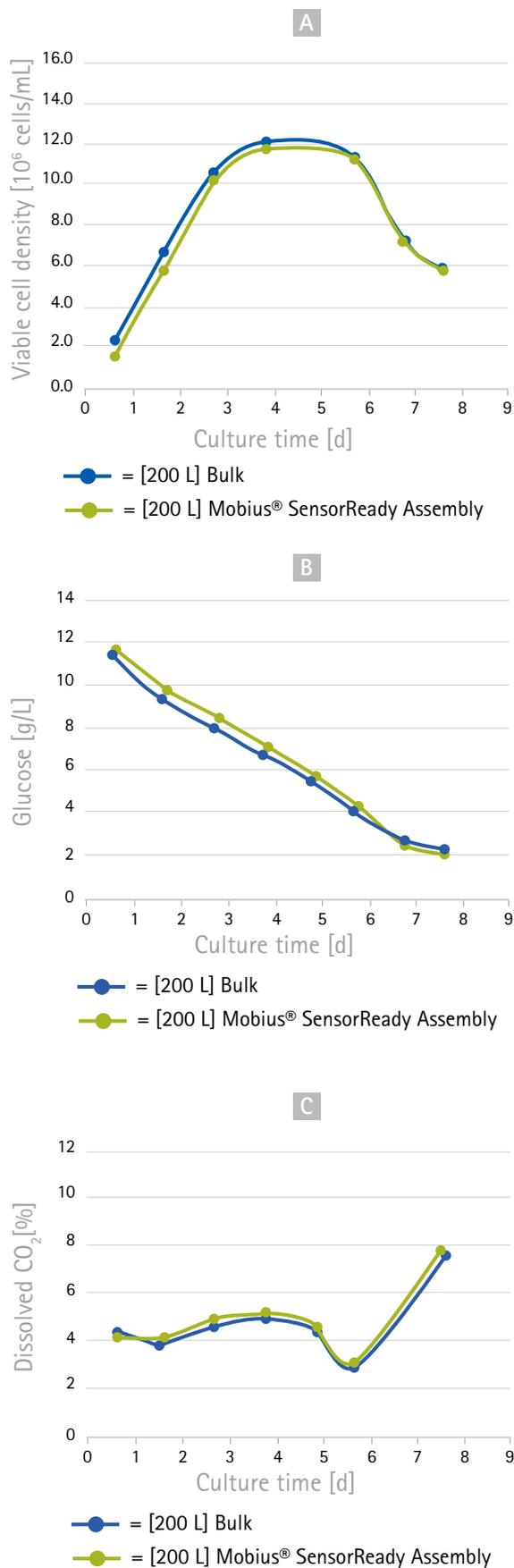


Figure 1B.

Mixing in 200 L Mobius® CellReady bioreactor process container (200 L volume). Each bar is an average (n=15). The error bars indicate 1 standard error from the 15 readings of T_{95} .

- Across the entire range of agitation rates tested, the Mobius® SensorReady mixing time was consistently representative of the mixing time observed at the other locations in the system. At the low and middle agitation rates, mixing time in the Mobius® SensorReady was comparable to the bulk mixing average. At high agitation rates (when mixing times are fastest), the mixing time observed within the Mobius® SensorReady loop may be a slight overestimation of the average bulk mixing time, as it more closely represents the mixing time observed at the top of the tank.
- In the 50 L bioreactor process container, the system average mixing times decrease from approximately 38 seconds to 24 seconds as agitation rates increase from 106 rpm to 192 rpm.
- In the 200 L bioreactor process container, the system average mixing times decrease from approximately 33 seconds to 26 seconds as agitation rates increase from 40 rpm to 110 rpm.
- These results indicate that the Mobius® CellReady 50 L and 200 L bioreactor process containers provide well-mixed conditions across a wide agitation operating window.





In support of the conductivity profiles generated in Figure 1, which show that the mixing time in the bulk is comparable to the mixing time in the Mobius® SensorReady loop, a second experiment was executed at the 200 L scale to demonstrate that the contents in the loop are continuously representative of the contents in the bulk bioreactor for the cell culture application. A sampling port was fitted on the bioreactor process container to enable samples to be drawn directly from the bulk to allow for subsequent comparison to samples drawn from the sample lines located on the Mobius® SensorReady assembly. An eight-day, batch cell culture process was performed in the Mobius® CellReady 200 L bioreactor process container while samples were drawn daily from both sample port locations.

- Samples drawn from the Mobius® SensorReady module are representative of the bulk contents for cell counts, media composition, and dissolved gas concentrations as shown in the viable cell density (Panel A), residual glucose (Panel B), and dissolved carbon dioxide profiles (Panel C).
- The cell culture environment within the loop is comparable to the cell culture environment in the bulk in terms of density, metabolites, and dissolved gas concentrations throughout the entire process.
- Cell culture sampling, process monitoring and process control are accurate and effective through the use of the Mobius® SensorReady assembly.
- Sampling from the Mobius® SensorReady loop requires minimal purging and eliminates the tendency for cells to settle near the sample location. In contrast, it was difficult to get an accurate cell density count from the specially made sample port from the bulk due to cell debris that persistently collected in the line. This debris was clearly visible even after purging, in the early days of the culture, samples drawn without excessive purging, and physical manipulation of the sample line may have contributed to slight overestimations of the actual cell densities (Figure 2 Panel A).

Figure 2.

Comparison of daily viable cell density (panel A), residual glucose (panel B), and dissolved carbon dioxide profiles (panel C) for samples drawn from the Mobius® SensorReady loop and the bulk sample port created. Samples were analyzed on the Vi-CELL® XR, (Beckman Coulter), BIOPROFILE® FLEX system (NOVA Biomedical), and blood gas analyzer (Siemens Rapidlab® 248).

Power

Calculating power per unit volume is a common approach used for process comparison and for scaling across bioreactors of varying geometries and designs. In this calculation, an impeller power number, unique to the impeller design, is used to determine the amount of energy that is dissipated into the cell culture through the rotation of the impeller blades. Using software unique to the motor, drive currents were recorded at different volumes and agitation rates, and used in conjunction with the motor torque constant (also unique to the motor) to determine the power (expressed in watts) required to turn the impeller in the liquid. For each study, the average of the calculated values determined the reported power number and the Reynolds number always exceeded 10,000. The wattages determined in the absence of any liquid volume (blades turning in air, water only in the impeller cup) were subtracted from the wattages determined at the varying agitation rates and volumes to ultimately determine the impeller power number. The impeller power number is then used to plot the energy dissipation rate at various volumes and agitation rates typically used in the Mobius® CellReady 50 L and 200 L bioreactor process containers, as shown in Figure 3A and 3B.



Impeller

The single, up-pumping bottom mounted, pitched-blade impeller (offset 15° from center), utilizes Levitronix® magnetically levitating, bearingless technology. A single, flexible baffle is designed to improve overall mixing. The 5:1 turndown ratio maximizes flexibility in operating volume, allowing the systems to be used as both seed and production vessels (200 L shown).

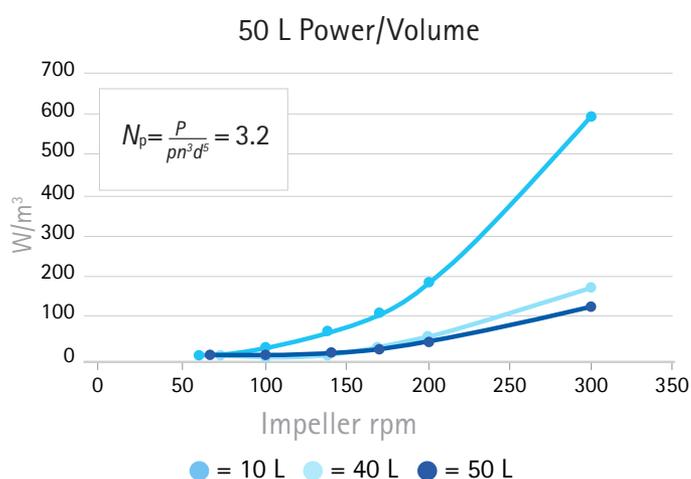


Figure 3A.

Power/volume for a range of agitation rates from 60 to 300 rpm across the min (10 L) and max (50 L) working volumes.

NOTE: the recommended operating range for agitation is 0 – 300 rpm.

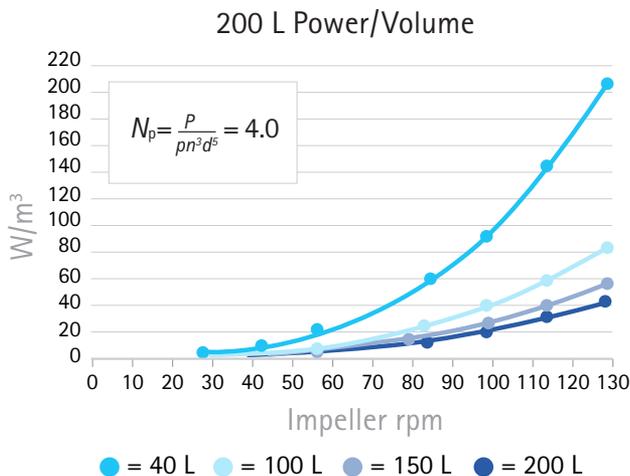


Figure 3B.

Power/volume for a range of agitation rates from 30 to 130 rpm across the min (40 L) and max (200 L) working volumes.

NOTE: the recommended operating range for agitation is limited to 30 – 120 rpm.

Volumetric mass transfer coefficient for Oxygen ($k_L a$)

One of the most critical features of a bioreactor is its ability to facilitate process gas exchange to provide an optimal environment for cell growth and production. Mass transfer capabilities for both 50 L and 200 L bioreactor process containers were characterized using the static gassing out method to determine the volumetric mass transfer coefficient ($k_L a$) for oxygen. Each $k_L a$ data point shown in Figures 4–6 was determined in a mock media buffer (1x PBS, 2 g/L Pluronic® F68 and 50 ppm Antifoam C) at 37°C. Experiments were performed by first sparging nitrogen to insure that the dissolved oxygen concentration was less than 2% air saturation prior to sparging air at the desired rate through the microsparger. Data were collected using a Finesse TruDO® electrochemical dissolved oxygen (DO) probe that had been installed in the Mobius® SensorReady Quad RS module (in some cases a Mobius® SensorReady SUS was used in series as well). The DO probe was calibrated to 100% air saturation prior to the experiments and no overlay gas was applied. The data shown in Figure 4 represent

triplicate determinations performed randomly. The $k_L a$ values shown represent the slope of the line created by plotting $\ln((C^*-C_{t_1})/(C^*-C_{t_2}))$ vs. time (t_2-t_1), where C^* is fully saturated liquid, C_{t_1} is percent air saturation at time zero, C_{t_2} is percent air saturation at time 2, and t_1 and t_2 are time zero and time 2 respectively. The slope is determined from a standard linear portion of the DO vs. time curve defined from 10 or 20% to 80 or 90% air saturation for each $k_L a$ value shown in Figures 4, 5, and 6.

- $k_L a$ increases with increasing gas flow across the entire range of agitation rates tested.
- Increasing agitation rate will increase $k_L a$ when operating at higher gas flow rates, while at low gas flow rates the effect of agitation rate on $k_L a$ is minimized.
- $k_L a$ values achievable in the Mobius® CellReady 50 L bioreactor process container at full operating volumes range from 3–66 hr⁻¹.
- $k_L a$ values achievable in the Mobius® CellReady 200 L bioreactor process container at full operating volume range from 5–60 hr⁻¹.

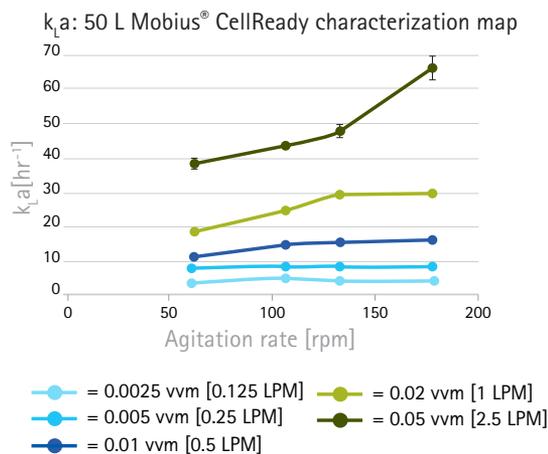


Figure 4A.

$k_L a$ map for 50 L working volume at various agitation rates and gas flow rates through the microsparger using mock media buffer. Each data point is an average (n=3). Error bars indicate one (1) standard deviation from the three (3) readings.

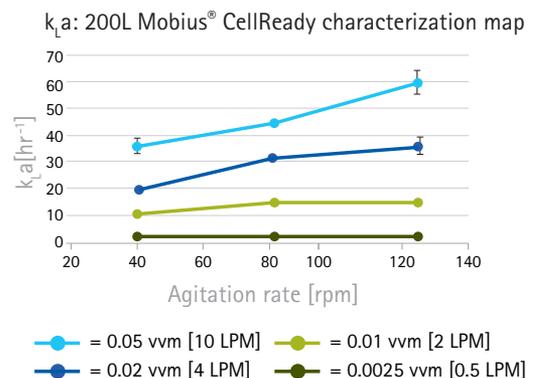


Figure 4B.

$k_L a$ map for 200 L working volume at various agitation rates and gas flow rates through the microsparger using mock media buffer. Each data point is an average (n=3). These experiments were performed in 3 different bioreactor process containers. Error bars indicate one (1) standard deviation from the three (3) readings.

The Tyvek® membrane microsparger located beneath the impeller is covered with a screen and over-molded into the plastic sparge plate to result in a sparger with strength, robustness and superior durability. Figure 5 shows a plot of $k_L a$ values obtained over the course of 500+ hours where gas flow was varied between 2 LPM and 10 LPM. Figure 6 shows a plot of $k_L a$ values obtained at various working volumes. Data shown in Figure 5 and 6 were gathered from the Mobius CellReady 200 L bioreactor process container.

- The Tyvek® membrane microsparger does not have a defined pore size; actual bubble size will vary depending upon the gas flow rate employed and the media used within the bioreactor.
- Sustained microsparger durability is achieved such that $k_L a$ (and therefore bubble size) are consistent and repeatable for over 500 hours of continuous sparging at both 2 LPM and 10 LPM air flow rates due to the robust microsparger design.
- The robust sparger design enables a wide range of suitable $k_L a$ values for supporting high oxygen demanding cell culture processes across the entire 40 L – 200 L working volume range.
- $k_L a$ values increase with decreasing volume when agitation rate (RPM) and gas flow rate (LPM) remain constant.
- $k_L a$ values are similar across the span of operating volumes from 40 L to 200 L when agitation rate and sparge gas flow rate are scaled by power per volume (W/m^3) and volume of gas per volume of liquid (vvm), respectively.

Optimal gas management in a bioreactor often includes the ability to remove excess CO_2 at certain times during the culture. To demonstrate the utility of the 2.3 mm open pipe sparger of the Mobius® CellReady 200 L bioreactor process container for CO_2 stripping, the CO_2 level of a day 10 cell culture was deliberately increased to 18% by sparging CO_2 gas into the culture. Air was then sent through the open pipe sparger at 0.5 LPM and the pH and CO_2 levels were monitored in samples taken through the Mobius® SensorReady sample ports using a blood gas analyzer (Siemens Rapidlab® 248) over a 6 hour period. The data shown in Figure 7 demonstrate that the open pipe sparger incorporated into the Mobius® CellReady bioreactor process container can be used to remove excess CO_2 from cultures if needed.

- The rate of pH change observed at 0.5 LPM air through the open pipe sparger was 0.05 units per hour.
- It should be possible to increase the rate of CO_2 stripping by using higher gas flow rates through the open pipe sparger.
- The open pipe sparger serves as a tool for stripping CO_2 during cell culture runs that require specific CO_2 concentrations.

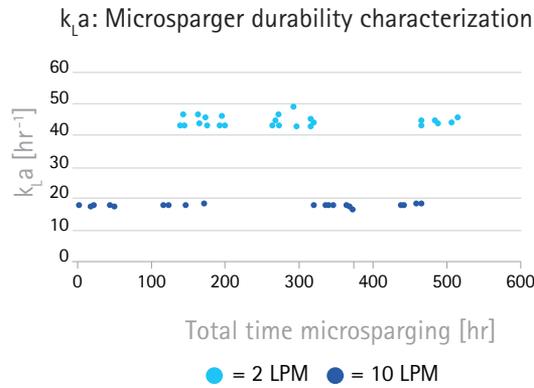


Figure 5. Mobius® CellReady 200 L bioreactor process container continuously operating at 81 rpm ($10 W/m^3$) for 21 days with gas flow rate oscillating between 2 and 10 LPM (n=53).

Microsparging durability data

Sparging Condition	2 LPM	10 LPM
Average $k_L a$ (hr^{-1}):	18.2 ± 0.3	44.7 ± 1.5
Coefficient of Variation (%)	1.4	3.3

$k_L a$ characterization across working volume

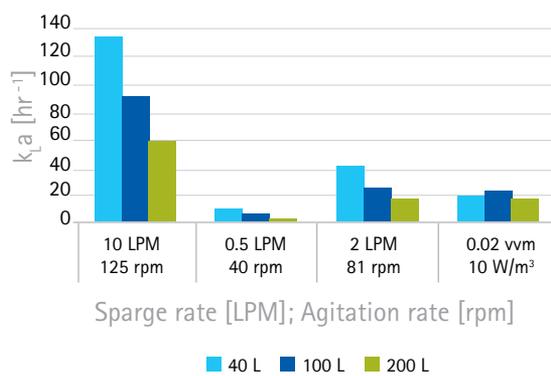


Figure 6. $k_L a$ characterization across the Mobius® CellReady 200 L bioreactor process container design space (n=1). $k_L a$ values were determined for high gas flow with high agitation rate, low gas flow with low agitation rate, 81 rpm with 2 LPM gas flow rate, and at the varying agitation rates and gas flow rates that equal $10 W/m^3$ and 0.02 vvm for 40 L, 100 L and 200 L working volumes using mock media buffer.

Stripping of CO_2 through open pipe

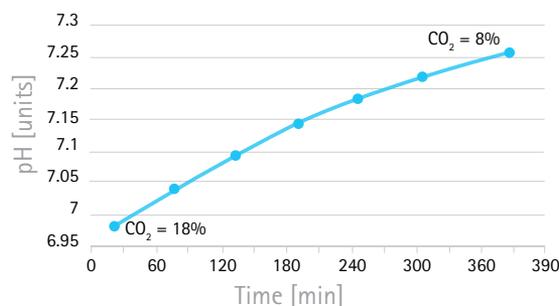


Figure 7. Demonstration of CO_2 stripping capability of the open pipe sparger during a 200 L cell culture trial.

Temperature control

The management of temperature is another critical requirement for successful cell culture. A series of experiments were performed to track the temperature profiles of the fully-jacketed Mobius® CellReady system. The experiments were designed to simulate typical application scenarios. The ability to heat 50 L and 200 L of media brought from cold room storage (e.g., 4 °C) or room temp (20 °C) to a normal operation temperature (37 °C) was examined. The system's capability to decrease the culture from a 37 °C to a 30 °C set point, simulating an in-process temp shift, was similarly tested. The specific controller PID settings, system control loop configuration, and temperature control unit chosen will impact these results.

- These data are examples of the bioreactor systems' heating and cooling capabilities and demonstrate that the control loop configuration parameters have a significant impact on heating/cooling time and overshoot.
- The Mobius® CellReady 50 L and 200 L fully jacketed systems are capable of heating their respective maximum working volumes of aqueous media from 4 °C to 37 °C in less than 5 hours with no detectable overshoot.
- The Mobius® CellReady 50 L and 200 L fully jacketed systems are capable of heating their respective maximum working volumes of aqueous media from 37 °C to 30 °C in 2 hours with no detectable overshoot.
- The system's liquid jacket extends across the bottom of the vessel to increase heating capabilities. Results for the 10 L and 40 L minimum working volumes show a slightly longer heating time for the PID loops tested. This is likely a result of slightly slower heat transfer occurring through the bioreactor process container hard base that makes up the majority of the liquid contact at this working volume.

Mobius® CellReady 50 L temperature control

TCU Unit	Volume (L)	Temperature Range (°C)	Stable to 0.2 °C (hr)	Overshoot (°C)
T1200	10	4 to 37	4.2	0.7
	50	4 to 37	1.8	0.5
	10	37 to 30	1.6	0.7
	50	37 to 30	0.6	0.3
RE630	10	4 to 37	4.3	0
	50	4 to 37	4.4	0.4
	10	37 to 30	2.2	0
	50	37 to 30	1.9	0.5

Mobius® CellReady 200 L temperature control

Volume (L)	Temperature Range (°C)	PID	Stable to 0.2 °C (hr)	Overshoot (°C)
40	4 to 37	PID A	6.5	0.0
200	4 to 37	PID B	4.8	0.0
200	4 to 37	PID C	5.0	0.0
200	4 to 37	PID D	5.4	1.2
200	20 to 37	PID E	3.5	0.4
40	37 - 30	PID A	1.6	0.0
200	37 to 30	PID B	2.0	0.0
200	37 to 30	PID C	2.0	0.0
200	37 to 30	PID D	2.0	0.0
200	37 to 30	PID F	2.8	0.0

Table 1.

In these studies, two temperature control units (Lauda® T1200 and Lauda® RE630) and a Finesse TruViu® control system were used. The PID settings were provided by Finesse Solutions LLC.

Table 2.

In these studies, a standard temperature control unit (Lauda® 1200) and Finesse TruViu® control system were used. Each PID loop (A-F) varied in just one control parameter, where PID A-C incorporated gain scheduling.

Cell culture and the Mobius® SensorReady pump

Flow through the Mobius® SensorReady assemblies is mediated by a Levitronix® bearingless, levitating, magnetic impeller in a polycarbonate housing that has been designed specifically for low shear applications. To demonstrate that this configuration does not negatively impact cell growth and viability, small-scale cell cultures were employed as test beds. Two 3 L bioreactors were configured with an external loop containing the Mobius® SensorReady pump. Bioreactors were inoculated at a 2 L working volume with CHO cells and for the duration of a ten-day batch culture, were continuously cycled through the loop at a 3 LPM fixed flow rate. A standard 3 L Mobius® CellReady bioreactor was inoculated in parallel to serve as the experimental (i.e., no pump) control.

- The 3 L bioreactors with the Mobius® SensorReady pumps had similar cell growth and viability profiles as the control bioreactor (Panels A and B).
- There was no significant difference in metabolism between the control bioreactor and the bioreactors with the Mobius® SensorReady pumps (Panel C).
- Even at this exaggerated recycle rate of flow through the Mobius® SensorReady pumps (up to 100-fold), no significant impact on the cell culture behavior was observed.
- The Mobius® SensorReady pump is effective at providing a low shear, controlled flow rate through the Mobius® SensorReady loop, suitable for the cell culture application.

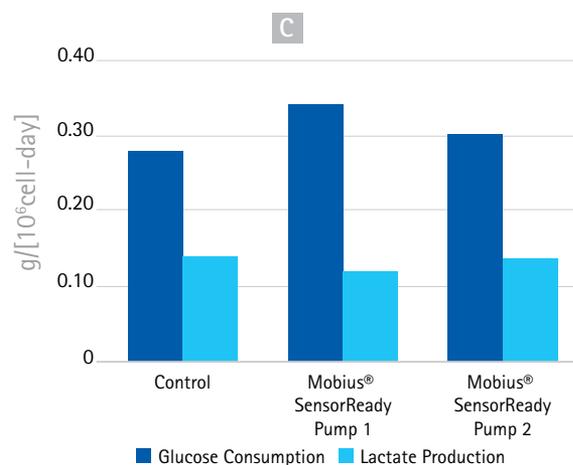
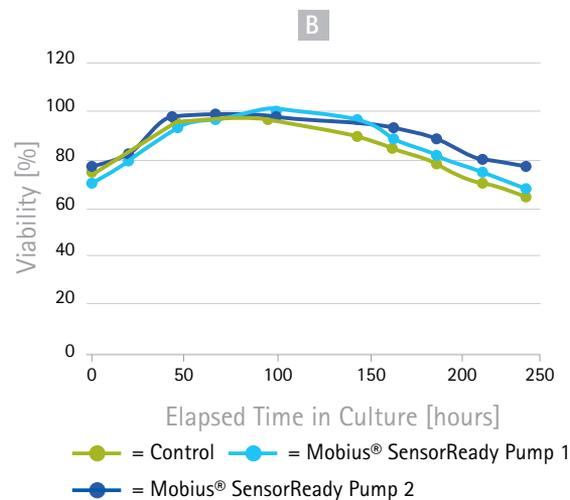
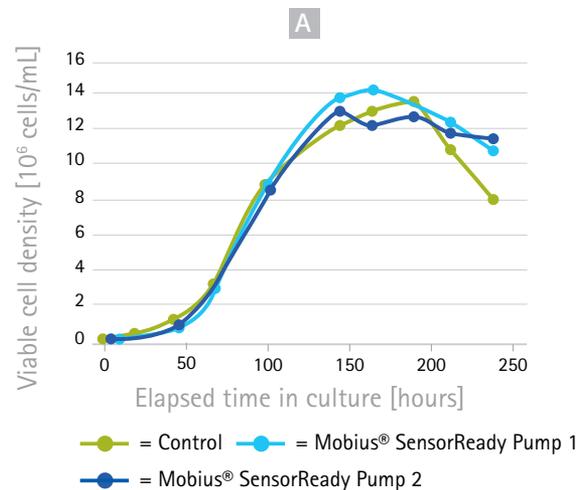


Figure 8.

The bioreactors were sampled daily and cell number and viability were determined using a Vi-CELL® XR (Beckman Coulter). Metabolite levels were determined daily using a BIOPROFILE® FLEX system (NOVA Biomedical). Glucose consumption and lactate production were calculated for days 1–4.

Mobius® CellReady 50 L and 200 L Bioreactor systems cell culture performance

A variety of cell culture performance experiments were conducted in the Mobius® CellReady 50 L and 200 L bioreactor systems. To demonstrate operating capability at minimum and maximum working volume, a CHO cell batch-culture process was inoculated as an N-1 step targeting 0.2×10^6 cells/mL at the minimum working volume. Growth characteristics were recorded for a five day period before cells were removed and fresh media was added to achieve the 0.2×10^6 cells/mL target inoculation density at the maximum working volume. The same lot of media was used for N-1 and production, the controller set points of pH = 7, DO = 30% air saturation and temp = 37 °C were standard across both steps, and the power/volume was maintained at 14 W/m^3 for each working volume tested.

Day 1-5 growth rates (μ):

50 L	200 L
10 L = 0.024 hr^{-1}	40 L = 0.032 hr^{-1}
50 L = 0.029 hr^{-1}	200 L = 0.032 hr^{-1}

Comparable cell growth performance characteristics can be achieved within the 1:5 working volume capacity from 40 L to 200 L in the 200 L; and from 10 L to 50 L in the 50 L.

A series of experiments were executed at the 50 L scale using one CHO cell line and at the 200 L scale using 2 different CHO cell lines to evaluate performance. Cells were scaled from a vial thaw, through shake flasks and Mobius® CellReady 3 L bioreactors. No more than one 3 L bioreactor was used to inoculate the N-1 Mobius® CellReady 50 L system at the 10 L working volume and no more than three 3 L bioreactors were used to inoculate the N-1 Mobius® CellReady 200 L system at the 40 L working volume. After a 4-6 day growth period, cells were removed and fresh media was added to achieve the 0.2×10^6 cells/mL target inoculation density at the production volume. For each 50 L and 200 L batch, a Mobius® CellReady 3 L bioreactor was welded to the Mobius® SensorReady sample line and filled with 2 L of day 0 production culture. The small scale satellite bioreactors were controlled at the same process set points of pH = 7, DO = 30% air saturation and temp = 37 °C. Power/volume was consistent at 14 W/m^3 across scales. Samples from the 50 L and 200 L production bioreactor process containers and the 3 L satellites were analyzed daily on the Vi-Cell® XR (Beckman Coulter) and the BIOPROFILE® FLEX system (NOVA Biomedical). The results are recorded in Tables 3 and 4 and shown in Figures 9A and 9B.

Figure 9A.

Viable Cell Density and Viability vs. culture time for a batch culture process operated at the 10 L and 50 L working volumes. Data was obtained from the Vi-CELL® XR (Beckman Coulter).

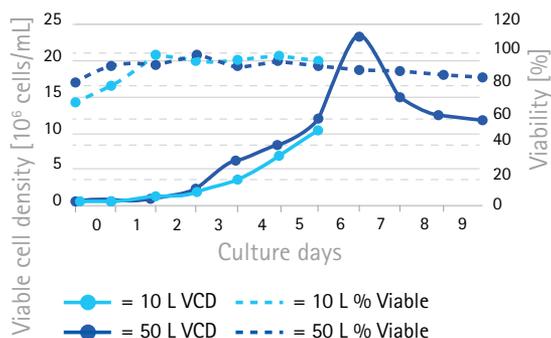
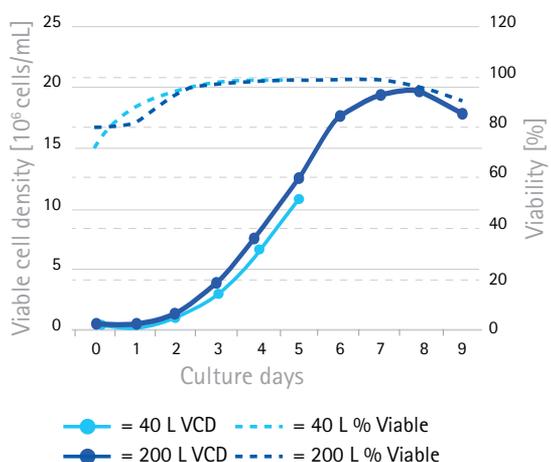


Figure 9B.

Viable Cell Density and Viability vs. culture time for a batch culture process operated at the 40 L and 200 L working volumes. Data was obtained from the Vi-CELL® XR (Beckman Coulter).



Mobius® CellReady 50 L production with Mobius® CellReady 3 L satellites

Experiment	Volume [L]	Growth Rate [1/hr]	Peak VCD [10^6 cells/mL]	Day 7 [% viable]	Peak Lactate [g/L]
Experiment 1	50	0.031	27	97	2.2
	2	0.033	23	90	2.2
Experiment 2	50	0.029	21	85	NA
	2	0.029	23	91	NA

Table 3.

50 L production culture growth rates, peak viable cell density, day 7 viability and peak residual lactate values are compared to 3 L satellite control bioreactors for one CHO cell line.

Mobius® CellReady 200 L production with Mobius® CellReady 3 L satellites

Cell Line	Volume [L]	Growth Rate [1/hr]	Peak VCD [10^6 cells/mL]	Day 7 [% viable]	Peak Lactate [g/L]
Cell Line 1 (Exp.1)	200	0.029	23	90	3.4
	2	0.025	22	95	3.3
Cell Line 1 (Exp.2)	200	0.028	22	95	2.7
	2	0.026	22	97	2.0
Cell Line 2	200	0.032	19.8	98.8	2.9
	2 (historical)	0.027	18.1	91.6	1.9

Table 4.

200 L production culture growth rates, peak viable cell density, day 7 viability and peak residual lactate values are compared to 3 L satellite control bioreactors for two different CHO cell lines.

Growth rate, peak density, viability, and peak lactate production profiles in the Mobius® CellReady 50 L and 200 L bioreactor systems are consistently comparable to the performance observed in satellite bioreactors and historical values at the 3 L scale, as demonstrated for 2 different CHO cell lines.

Conclusion

Successful bioprocessing relies on the ability to accurately and effectively monitor and control critical process parameters. Bioreactor characterization properties such as mixing time, volumetric mass transfer capabilities, temperature mapping and power input define the process design space wherein accurate and effective monitoring and control can occur. In addition to physical characterization of the processing design space for the 10 L through 50 L working volume capacity for the 50 L Mobius® CellReady bioreactor system and 40 L through 200 L working volume capacity for the 200 L Mobius® CellReady bioreactor system, cell culture capabilities were demonstrated with the execution of cell culture processes achieving peak cell densities $> 20 \times 10^6$ cells/mL, with growth and metabolic behavior comparable to 3 L scale-down controls. The collective results of these studies confirm that the Mobius® CellReady 50 L and 200 L single-use bioreactor systems are capable of supporting a wide range of biomanufacturing processes.



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