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Application Note

Scalability from Mobius® 3 L Single-Use Bioreactor to 50 L – 2000 L Mobius® iFlex Bioreactors to support intensified upstream process development

Marisa Maher, Samantha Whitney, Jonathan Cain, Amy Wood, MilliporeSigma, Bedford, MA, USA

Introduction

Properly scaling from Mobius® 3 L Single-Use Bioreactors to Mobius® iFlex Bioreactors for intensified processes is highly dependent on maintaining mixing, oxygenation, nutrient, and process control. To be able to meet these criteria, bioreactor systems need to have a well-characterized design space while considering risks and ways to control hydrodynamic shear. Extensive understanding of the bioreactor design space and the environmental conditions experienced by the cells within the systems' operating range enables more informed process development and predictable performance across scales.

The Mobius® 3 L Single-Use Bioreactor and the 50-2000 L Mobius® iFlex Bioreactors are designed for efficient cell growth of batch, fed batch, and perfusion upstream processes. The bioreactors are optimized for high volumetric mass transfer (k_La) and power input (W/m^3) with low mixing time while balancing shear limits through impeller tip speed and sparger bubble size.

In this study, key parameters of power input, power number, mixing time, tip speed and shear calculations for the Mobius® 3 L Single-Use Bioreactor and the 50-2000 L Mobius® iFlex Bioreactor impeller designs were characterized. Additionally, k_La and bubble size from all available sparger designs were characterized to help understand bubble shear impact when scaling from 3 L to Mobius® iFlex Bioreactors. Lastly, CO_2 stripping efficiency was assessed to understand trends per sparger type during scale-up.

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Geometric Scalability and Dimensions

Bioreactor vessel design impacts mixing performance, oxygen transfer rate, power per volume, power number, and heat transfer. Since these parameters have a significant influence on the cell culture process, successful transfer of a cell culture process across scales first requires careful consideration of the vessel design. Therefore, to make processes more easily scalable across sizes, vessel geometries are designed to maintain similarity from bench to commercial scale. The aspect ratio (H:D) of a bioreactor compares the vessel height to diameter geometry and the turndown ratio dictates the acceptable minimum working volume at each vessel scale. The 1.8-2.0:1 H:D ratio follows typical aerobic cell culture vessel designs which balance gentle, homogeneous mixing, varying working volumes, and effective mass transfer of gasses in and out of the cell environment. The 5.0:1 turn down ratio in the 200 L – 2000 L systems are enabled by the bottom mounted impeller design and allow for greater flexibility in operating volume which offers a lower range of available volumes while ensuring the impeller is fully submerged during operation. The 3 L and 50 L vessels have slightly higher minimum operating volumes to fully submerge the impeller, sensors, ports, and in the case of the 3 L to achieve proper heating with a heating blanket.

Table 1. Mobius® Bioreactor designs

	3 L	50 L*	200 L	500 L*	1000 L*	2000 L*
H:D	1.8:1	2.0:1	2.0:1	2.0:1	2.0:1	2.0:1
Maximum Working Volume (L)	2.4	50	200	500	1000	2000
Minimum Working Volume (L)	1	15	40	100	200	400
h_{fmax}:H	0.8:1	0.8:1	0.8:1	0.8:1	0.8:1	0.8:1
h_{fmin}:h_{fmax}	2.7:1	3.3:1	5.0:1	5.0:1	5.0:1	5.0:1
di:D	0.55	0.34	0.38	0.35	0.34	0.35
Vessel Diameter (cm)	13.7	34.0	54.6	72.9	91.9	115.8
Impeller Geometry	Up-pumping marine (3 blades)	Down-pumping pitched blade (4 blades)				
Impeller Position	On shaft, centered	Bottom mount, 15° from center				
Impeller Power Number	0.3	3.6	3.6	3.6	3.6	3.7
Baffles	N/A	X – Baffle				
Open Pipe Sparger Orifice (mm)	2.3	4.4	7.4	2 x 7.4	2 x 10.4	2 x 10.4
Drilled Hole Sparger	N/A	900 150 µm drilled holes in Ultimus® film	2250 150 µm drilled holes in Ultimus® film	4490 150 µm drilled holes in Ultimus® film	6740 150 µm drilled holes in Ultimus® film	8980 150 µm drilled holes in Ultimus® film
Microsparger/High Performance Sparger	15-30 µm Sintered polyethylene	14170 20 µm drilled holes in Ultimus® film	35430 20 µm drilled holes in Ultimus® film	70870 20 µm drilled holes in Ultimus® film	106300 20 µm drilled holes in Ultimus® film	141730 20 µm drilled holes in Ultimus® film

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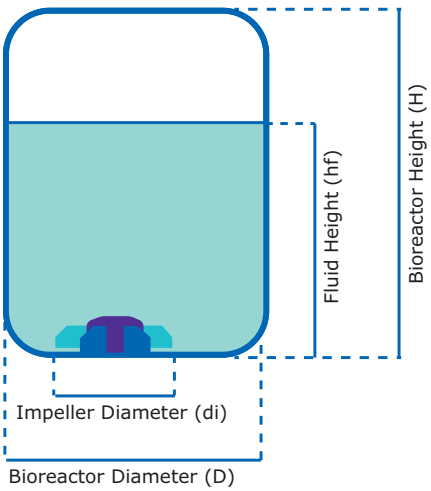

Figure 1. Key for bioreactor geometry in **Table 1**.

Impeller and Power

Power per volume describes the energy transfer 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.

Power and Shear Calculations

To calculate power density, 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 in order to improve predictability of cell culture performance at different volumes, should be constant across systems of different scales (**Eq. 1-5**). Power number is unique to impeller and motor designs. To support scalable performance in the Mobius® iFlex Bioreactors, the 50 L – 2000 L scale impellers were designed to maintain a constant power number for scalable power input and mixing performance. Although the Mobius® 3 L Single-Use Bioreactor has a different impeller geometry and power number compared to the Mobius® iFlex Bioreactors, power curves, tip speed, shear calculations, and overall mixing performance can be characterized to assess the design space and draw comparisons in the cell culture environment across scales.

Mixing Time Method

Mixing homogeneity and low mixing times are critical for providing a stable local environment for the cells and delivering efficient oxygenation and CO_2 stripping at any scale. To assess the mixing performance, experiments were conducted at maximum working volumes and power ranging from 10-100 W/m^3 in representative acrylic tanks of the Mobius® iFlex Bioreactors and in the Mobius® 3 L Single-Use Bioreactor. Several methods for determining mixing times including sensor and colorimetric mixing methods reported in industry were compared. The use of a combined approach including a pH sensor method and phenolphthalein colorimetric method was determined best fit for characterization based on accuracy, utility, and ease of use.[1] Two pH probes were placed within the 200 L - 2000 L tanks and response curves were recorded following the addition of concentrated acid or base; for the 3 L and 50 L scale, only one pH probe was used due to space constraints.

At the start of the experiment, the tank was filled with a solution of DI water dyed with 1 ppm of phenolphthalein and was then adjusted to a starting pH of 4.0 using HCl. Solutions of 5M NaOH and HCl were then added to the center of the tank alternately at a ratio of 1 mL per 25 L of solution, allowing tank conditions to stabilize between each addition. Mixing time was determined as the time for the pH profile to reach 99% of the final resting value at each agitation rate tested. Each study was recorded using a video camera to gain additional information about mixing patterns and homogeneity as a pH-dependent colorimetric method was performed simultaneously with each pH sensor trial.

Mixing and Power Results

Both Mobius® 3 L Single-Use Bioreactor and Mobius® iFlex Bioreactor impeller designs can achieve >90 W/m^3 with <2.2 m/s tip speed, and less than a 34 second mixing time at full volume and maximum power density. Mobius® iFlex Bioreactors were designed with an off-center, bottom-mounted impeller and a novel internal baffle design to deliver a homogeneous mixing pattern as determined by the colorimetric visual observation. The mixing pattern in the Mobius® 3 L Single-use Bioreactor which utilizes a marine style impeller mounted on a shaft also was observed to deliver good mixing homogeneity where mixing time is minimal. For estimated shear rates (**Eq. 7-8**), the maximum calculated is 689 s^{-1}, which is referenced to be below critical shear rates for mammalian cell culture.[2]

Table 2. Impeller performance and shear estimations (Eq. 1-8)

Bioreactor Scale	Volume (L)	P/V (W/m³)	RPM	Tip Speed (m/s)	Re	Tip Shear Rate (1/s)	Cup Shear Rate (1/s)
3 L	Min (1 L)	10	141	0.56	15,000	63.3	N/A
(N_p: 0.3)		50	241	0.96	26,000	108	N/A
		100	304	1.21	33,000	136	N/A
	Max (2.4 L)	10	189	0.75	20,000	85	N/A
		50	323	1.29	35,000	145	N/A
		95	400	1.60	43,000	179	N/A
50 L*	Min (15 L)	10	74	0.45	19,000	22.8	209
(N_p: 3.6)		50	126	0.77	32,000	39.0	357
		100	158	0.97	40,000	49.1	450
	Max (50 L)	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	Min (40 L)	10	39	0.43	32,000	12.9	185
(N_p: 3.6)		50	67	0.73	54,000	22.0	316
		100	84	0.92	69,000	27.7	399
	Max (200 L)	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*	Min	10	38	0.51	46,000	14.3	122
(Np: 3.6)	(100 L)	50	66	0.87	78,000	24.4	209
		100	83	1.10	99,000	30.8	263
	Max	10	66	0.87	78,000	24.4	209
	(500 L)	50	112	1.49	134,000	41.8	357
		100	141	1.88	169,000	52.6	450
1000 L*	Min	10	34	0.56	62,000	14.7	154
(N_p:3.6)	(200 L)	50	59	0.96	106,000	25.2	264
		100	74	1.21	133,000	31.7	333
	Max	10	59	0.96	106,000	25.2	264
	(1000 L)	50	101	1.64	180,000	43.0	451
		100	127	2.07	227,000	54.2	569
2000 L*	Min	10	28	0.59	85,000	11.3	124
(N_p: 3.7)	(400 L)	50	47	1.01	145,000	19.3	213
		100	60	1.27	183,000	24.3	268
	Max	10	47	1.01	145,000	19.3	213
	(2000 L)	50	81	1.72	248,000	32.9	363
		100	102	2.17	312,000	41.5	458

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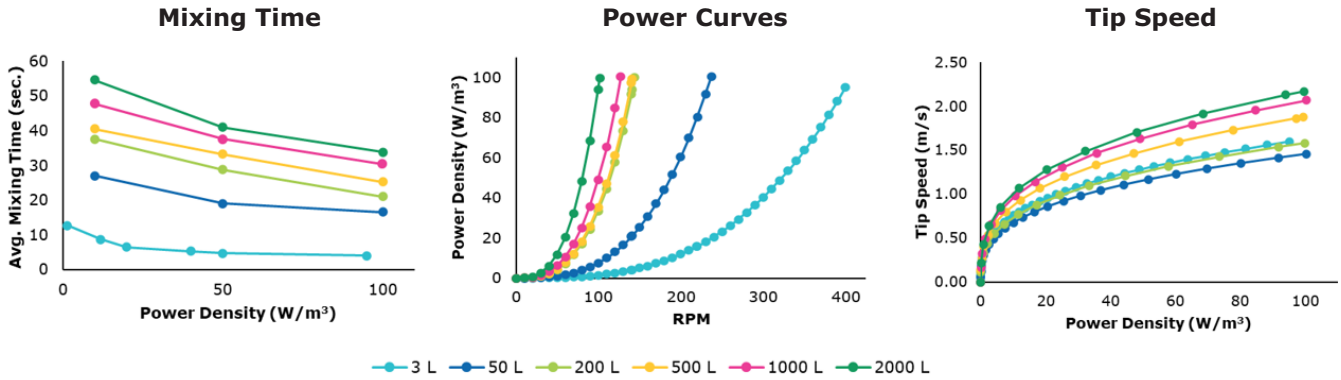


Figure 2. Mixing time per power density from 3 L to 200 L at full volume. Designs achieve less than 34 sec mixing time at maximum power density.

Figure 3. Power curves from 3 L to 200 L. Each reach > 90 W/m³ within operating range of rpm at full volume.

Figure 4. Tip speed per power density 3 L to 2000 L. All impellers achieve <2.2 m/s at >90 W/m³ at full volume.

Volumetric Mass Transfer (k_La)

Achieving sufficient oxygen transfer using air and/or pure oxygen is a key scale up parameter which can affect CO_2 accumulation, cell growth, viability, metabolism, and protein production. To achieve scalable mass transfer performance between bench scale (3 L) and large scale (50 L – 2000 L) there must be adequate impeller, mass flow controller, and sparger designs to provide an overlapping design space which meets the process demands. Here, k_La was determined at both bench and large scale under similar operating ranges to map the design space, explore the effect of buffer on k_La performance and compare the theoretical viable cell density (VCD) each scale can support. Bubble size was also characterized for each sparger design to help control and assess the risk of bubble shear when transferring processes between scales and systems.

k_La Method and Theoretical VCD Estimation

k_La was determined via the static gassing out method. Dissolved oxygen (DO) sensors were calibrated with air at 100% saturation. Using nitrogen, the system was purged until the sensor reached <2% DO. Then, air was sparged until the sensor reached saturation. The k_La value for each trial was calculated from the DO vs time graph. To maintain consistency in determining the k_La, the calculation was based on DO concentrations of 10% to 90% of measured air saturation. The k_La values reported represent the slope of the line created by plotting **Eq. 13** versus time ($t-t_1$). The k_La values derived from the most representative mock media solution were used to approximate VCD by applying equations **11-12**.

Bioreactors were run at various conditions listed in **Table 3**. 3 L data is the average ± standard deviation (SD) of n=2 Mobius® 3 L Single-Use Bioreactors with one trial each at 2 L volume, and the 200 L data is the average ± SD of two CO_2 or DO probes run n=3 times per condition at 200 L volume.

Table 3. k_La Conditions

Conditions	3 L Scale	200 L Scale
Volume (L)	2	200
Sparger	Open pipe Microsparger	Open pipe Drilled-hole High performance
Solutions	• 1 X PBS • 1 X PBS 4 g/L EMPROVE® Poloxamer 188, 50 ppm Antifoam C Emulsion • EX-CELL® Advanced HD Perfusion Media, 4 g/L EMPROVE® Poloxamer 188, 50 ppm Antifoam C Emulsion	• 1 X PBS • 1 X PBS 4 g/L EMPROVE® Poloxamer 188, 50 ppm Antifoam C Emulsion
Power Input Per Volume (W/m³)	20 W/m³ (224 rpm) 100 W/m³ (383 rpm)	20 W/m³ (84 rpm) 100 W/m³ (144 rpm)
Flow Rate (SLPM)	0.01 0.2 0.5	2 10 20 50 (drilled-hole and high performance only)

k_La Results

3 L and 200 L k_La in mock media solutions

To compare the design space in the 3 L Mobius® Single-Use Bioreactor and the 200 L Mobius® iFlex Bioreactor system, k_La was characterized from 0.01 - 0.25 vvm and 20 - 100 W/m³ at 2 L volume in the 3 L Mobius® Single-Use Bioreactor and 200 L volume in the 200 L Mobius® iFlex Bioreactor. This allows for proper estimation of volumetric mass transfer capabilities for each sparger type and the resulting flow rate requirements at each scale across a range of operating conditions. For low and high power per volume values (20 W/m³ and 100 W/m³) and two different characterization buffers or mock media solutions (1X PBS supplemented with 4 g/L EMPROVE® Poloxamer 188, 50 ppm Antifoam C, and 1X PBS, respectively), the open pipe and microsparger/high performance sparger deliver similar k_La values across the scales. Included in the Mobius® iFlex Bioreactors, there is also a third sparger available, adding a mid-range option in between the open pipe and high performance/microsparger for both k_La and bubble size.

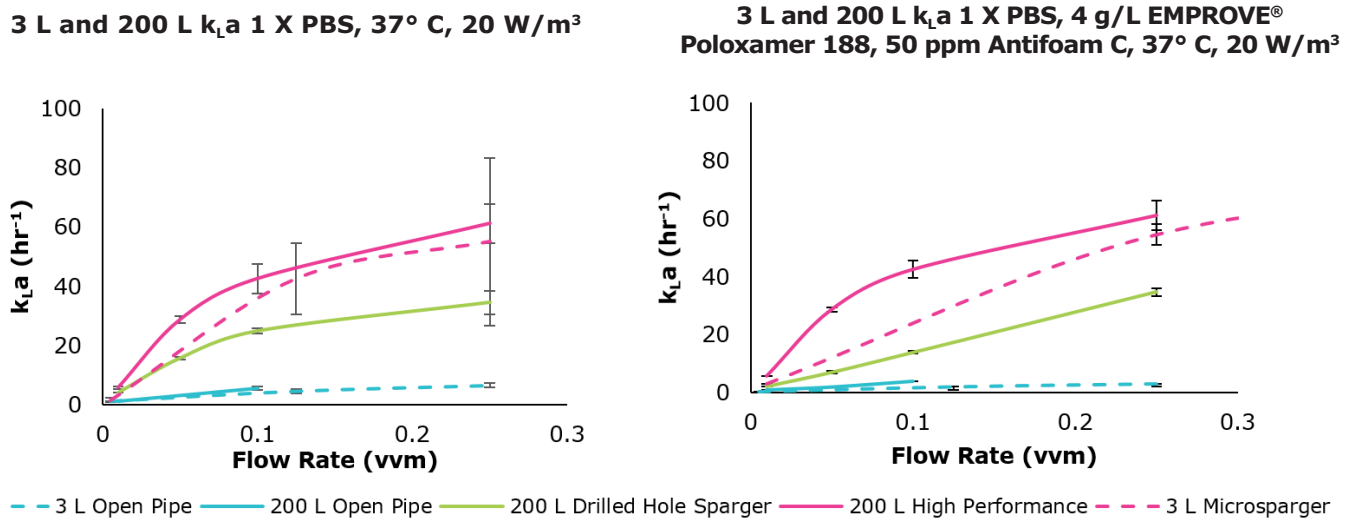


Figure 5. k_La characterization in two different mock media for open pipe, drilled-hole sparger, and high performance/microsparger at 3 L and 200 L scale at 20 W/m³. 3 L data is the average ± SD of n=2 Mobius® 3 L Single-Use Bioreactors and the 200 L data is the average ± SD of two CO_2 or DO probes run n=3 times per condition.

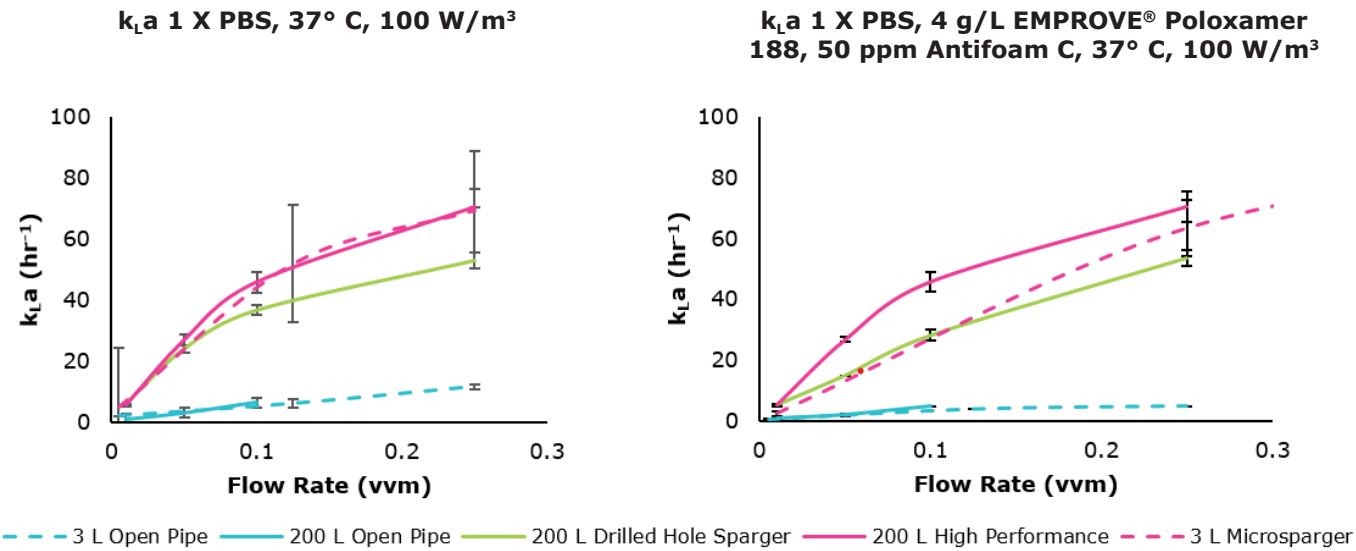


Figure 6. k_La characterization in two different mock media for open pipe, drilled-hole sparger, and high performance/microsparger at 3 L and 200 L scale at 100 W/m³. 3 L data is the average ± SD of n=2 Mobius® 3 L Single-Use Bioreactors and the 200 L data is the average ± SD of two CO_2 or DO probes run n=3 times per condition.

k_La comparison from mock media to EX-CELL® Advanced HD Perfusion Media to estimate theoretical VCD capacity

In order to accurately estimate theoretical viable cell density capabilities from a mass transfer perspective, understanding how the k_La performance in the mock media compares to the k_La performance in the specific cell culture medium is necessary. This is because the fluid properties and composition can impact the bubble size and gas mass transfer dynamics therefore changing the experimentally derived k_La value. However, characterization at large volumes becomes cost-prohibitive for standard cell culture media formulations, and lower-cost solutions may be desirable for these studies. Here we explore k_La characterization for each sparger design in two different characterization buffers or "mock media" solutions to assess which one is most representative of the performance in the intended cell culture medium.

For each sparger characterization:

- **Media**: EX-CELL® Advanced HD Perfusion Media supplemented with 4 g/L EMPROVE® Poloxamer 188 and 50 ppm EX-CELL® Antifoam C
- **Mock Solution 1**: 1 X PBS
- **Mock Solution 2**: 1 X PBS supplemented with 4 g/L EMPROVE® Poloxamer 188 and 50 ppm EX-CELL® Antifoam C

These data show that the buffer used for k_La characterization can have a significant effect on the reported k_La value, and that even for EX-CELL® Advanced HD Perfusion Media supplemented with 4 g/L EMPROVE® Poloxamer 188 and 50 ppm EX-CELL® Antifoam C, the aqueous solution that can deliver the most representative k_La value can vary depending on which sparger is being characterized. **Table 4** outlines the theoretical VCD that each system would be able to support based on in-depth k_La characterization across spargers, operating conditions, and buffers most representative of a specific cell culture media.

3 L Mobius® Open Pipe, 2 L Working Volume, 37°C

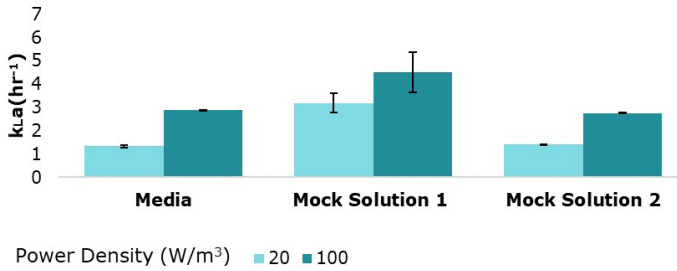


Figure 7. For the open pipe sparger, a 1 X PBS 4 g/L EMPROVE® Poloxamer 188 50 ppm EX-CELL® Antifoam C solution (Mock Solution 2) performs more similarly to media k_La, and therefore characterization data from this solution would be more appropriate to use to estimated VCD. Data is the average ± SD of n=2 Mobius® 3 L Single-Use Bioreactors.

3 L Mobius® Microsparger, 2 L Working Volume, 37°C

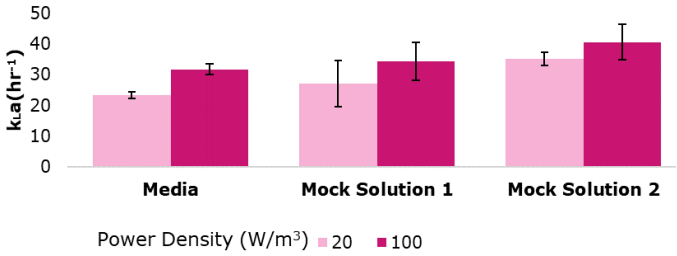


Figure 8. For the microsparger, a 1 X PBS solution (Mock Solution 1) is closest to the cell culture media k_La performance, and therefore characterization data from this solution would be more appropriate to use to estimate VCD. Data is the average ± SD of n=2 Mobius® 3 L Single-Use Bioreactors.

Table 4. Summary of sparger design and theoretical maximum VCD for both 3 L and 50-2000 L scales as derived from media-representative k_La values.

Sparger Design – 3 L	3 L Theoretical Max VCD*	Sparger Design – 50 L to 2000 L	200 L Theoretical Max VCD*
Open pipe (single drilled hole in plastic)	20 e^6 cells/mL	Open pipe (single drilled hole in plastic)	20 e^6 cells/mL
N/A		Mid-range drilled-hole (drilled holes in film)	120 e^6 cells/mL
Microsparger (sintered polyethylene)	300 e^6 cells/mL	High performance (drilled holes in film)	200-300 e^6 cells/mL**

*Using a cell specific oxygen consumption rate (qO_2) of 5 pmol/cell*day (moderate CHO consuming cell line) and most representative mock media per sparger for k_La values at 0.5 SLPM for 3 L, 20 SLPM for 200 L, 100% oxygen

** To reach a theoretical 300 e^6 cells/mL, 50 SLPM must be used at 200 L scale which is above the exit velocity shear target of 60 m/s and may increase foam production/accumulation.[2]

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.[3] To protect cells against bubble shear, poloxamers are typically included in bioreactor media in order to decrease surface tension and cause less damage to cells at burst. The offering of multiple sparger types with different bubble sizes gives options based on the bubble shear sensitivity of the cell line.

Bubble Size Characterization Method

Spargers were removed from the bioreactors and submerged in a 20 L rectangular tank. Media was imitated by using a high-salt concentration solution of Dulbecco's Phosphate Buffered Saline (1X PBS) and varying concentrations of Poloxamer 188 EMPROVE® EXPERT. A high-speed camera captured bubbles created by the sparger within the same frame as a reference measurement (a ruler). Using the software ImageJ, frames are converted into 7-10 images, and bubbles are traced in the same plane as the ruler, using it as a reference measurement within the software. 50-100 bubbles are traced per condition and Image J outputs minimum and maximum diameter of bubbles and converts to circular bubble diameter through **Eq. 10**.

Table 5. Bubble size test conditions.

Conditions	3 L Scale	200 L Scale
Volume (L)	20 L Rectangular Tank	
Sparger	Open pipe Microsparger	Open pipe Drilled-hole High performance
Solutions	• 1 X PBS • 1 X PBS 2 g/L EMPROVE® Poloxamer 188 • 1 X PBS 4 g/L EMPROVE® Poloxamer 188 • 1 X PBS 6 g/L EMPROVE® Poloxamer 188	
Power Input Per Volume (W/m³)	No Mixing	
Flow Rate (SLPM)	0.05 1	2 20

Bubble Size Results

Bubbles size delivered by each sparger in the Mobius® family of bioreactors in varying concentrations of poloxamer and at high and low gas flow rates has been characterized and is reported below. This information is critical to understanding the overlapping design space and local environment experienced by the cells when scaling across systems. Bubble size of the new open pipe and drilled-hole spargers in the Mobius® iFlex Bioreactors were designed to stay above the 2 mm bubble shear limits found in literature, and near the cutoff for the high performance sparger.[3] The spargers of the Mobius® iFlex Bioreactors are found to consistently deliver a greater bubble size than those delivered by the Mobius® 3 L microspargers to minimize risk of impactful bubble shear when transferring a process to the larger scale. For bubble shear sensitive cell lines, the Mobius® 3 L also offers an open pipe sparger with a larger bubble size. The Mobius® iFlex Bioreactor offers 3 bubble size options correlated to varying bubble shear risks that presents options from scaling from the Mobius® 3 L Bioreactor microsparger or open pipe. Risks based on bubble size is described in **Figure 11**. It is noted that entrance velocity (**Eq. 9**) and associated shear would increase with increasing flow rate through the Mobius® iFlex Bioreactor spargers, however, the drilled-hole and high-performance spargers were designed to deliver equivalent entrance velocity capped at 60 m/s when operating at the max operational flow rate through the sparger. The Mobius® iFlex Bioreactor high performance sparger will stay below 60 m/s velocity value at 40% of the maximum operating flow rate. This allows for a usable range of high performance sparger operation while providing additional area to explore with processes that are resistant to the effects of entrance velocity above the 60 m/s value.

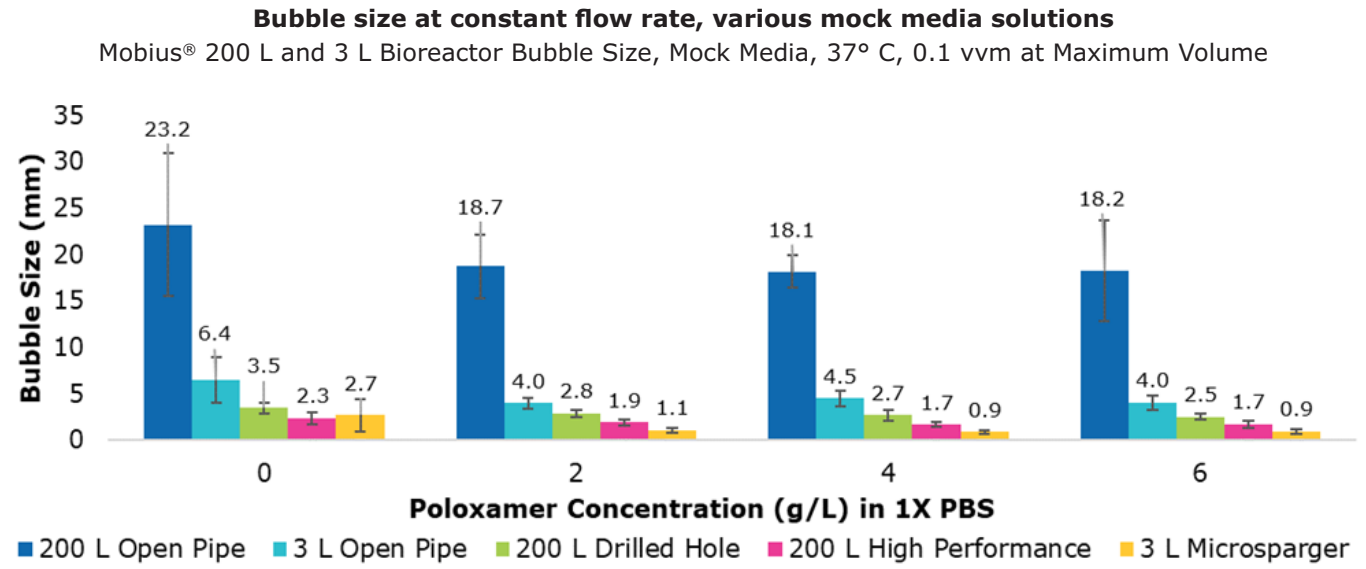


Figure 9. Bubble size characterization of newly designed Mobius® iFlex Bioreactor 50 L – 200 L spargers (open pipe, drilled-hole, high performance) compared to Mobius® 3 L Bioreactor spargers among several mock media formulations. Error bars are standard deviations.

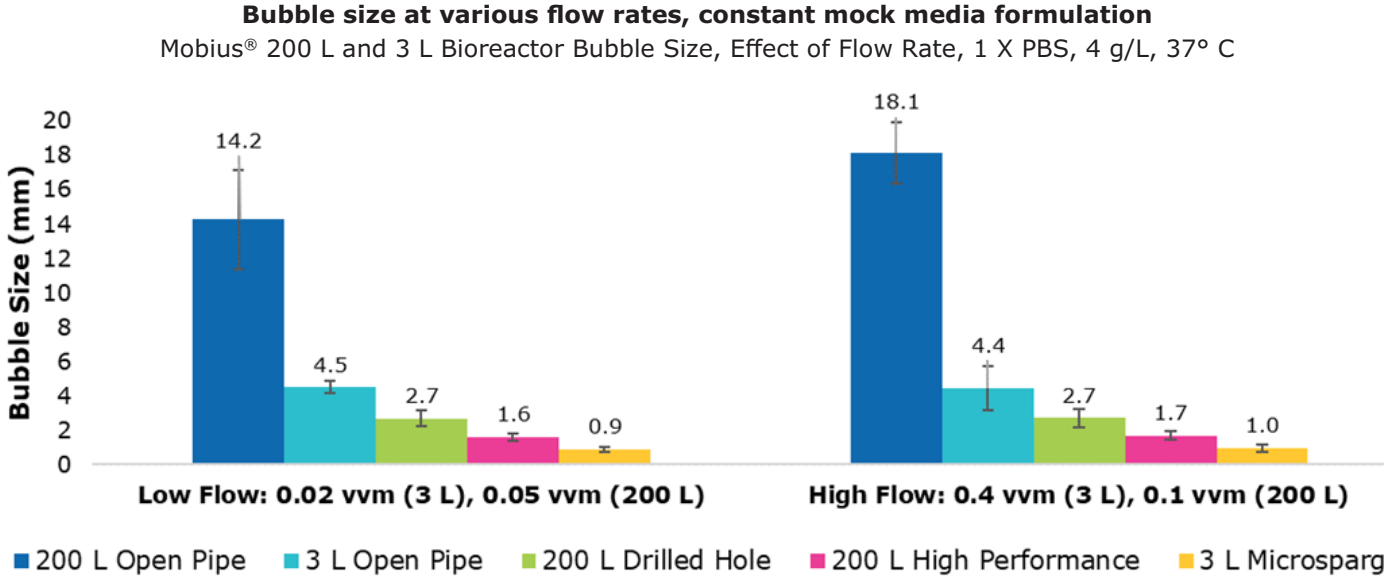


Figure 10. Results of high and low flow rate per scale at 1 X PBS, 4g/L EMPROVE® Poloxamer 188. Increase in bubble size for large scale open pipe, and minimal effect from flow rate seen for the remaining sparger. Error bars are standard deviations.

Figure 11 is a diagram to describe the theoretical bubble shear risk scaling from the 3 L Mobius® Single-Use Bioreactor to the 50 – 2000 L Mobius® iFlex Bioreactors. The top box in white describes the sparger type used in the 3 L Mobius® Single-Use Bioreactor process. Underneath, the color scale shows the risk of using Mobius® iFlex Bioreactor spargers, based on bubble size. For example, if a successful process is created using the 3 L microsparger, there is low bubble shear risk when scaling to the Mobius® iFlex Bioreactor, since the 3 L microsparger bubble size is smaller than all three Mobius® iFlex Bioreactor sparger types.

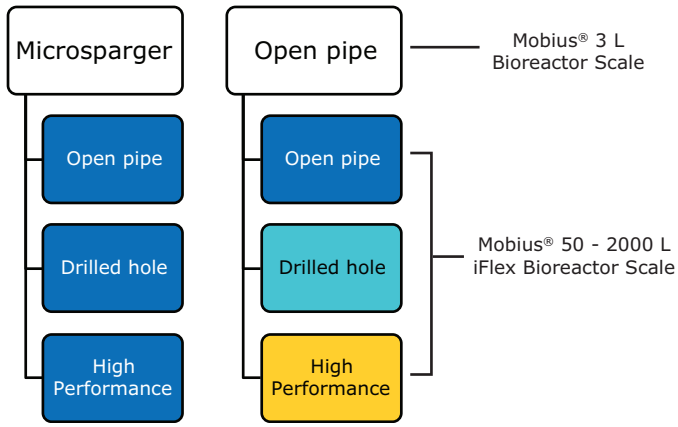


Figure 11. Heat map of bubble shear risk during scale up from a 3 L process, according to measured bubble size. blue = low risk, turquoise = medium risk, yellow = higher risk.

CO_2 Stripping

Higher cell densities generated by intensification result in increased oxygenation requirements and CO_2 generation for a given process. At larger scales, the increased bubble residence time and lower ratio of volume to liquid-headspace surface area can cause challenges with CO_2 accumulation, making consistent scale-up difficult. Maintaining appropriate CO_2 levels throughout a process is critical for control of pH and can have negative effects on cell growth and product quality if levels deviate from physiological conditions. This demonstrates the need to appropriately understand and manage sparging activities, for both oxygenation and CO_2 stripping to achieve optimal productivity and product quality during scale-up.

CO_2 stripping method and endpoints

The static gassing out method was used to determine a k_La value for air sparged into a CO_2 saturated solution. CO_2 was sparged into the system until saturation reached <10% DO. Air was then introduced via the sparger until the solution reached >90% DO. DO data was taken in a range of 10% to 90% and k_La was calculated.

A ratio of oxygenation k_La from sparging air into an N_2 saturated solution (results shown above) to CO_2 stripping k_La of air into a CO_2 saturated solution was determined to assess the spargers' stripping efficiency for the two gasses and relate values of oxygenation and CO_2 stripping capacity. CO_2 removal rate (%/hr) was calculated within a 10 – 5% CO_2 range to represent a typical cell culture environment for examining practical CO_2 stripping capabilities.

CO_2 Stripping Results

3 L and 200 L CO_2 stripping endpoints in mock media

CO_2 stripping endpoints of k_La into a CO_2 saturated solution and CO_2 removal rate (%/hr) were compared from the Mobius® 3 L Bioreactor to the Mobius® 200 L iFlex Bioreactor for similar sparger types at 20 W/m³. Mock media of 1 X PBS, 4 g/L EMPROVE® Poloxamer 188, 50 ppm Antifoam C Emulsion was used for comparison between scales. Results showed consistent performance per sparger type (open pipe to open pipe and microsparger to mid-range drilled-hole sparger) from 3 L to 200 L scale.

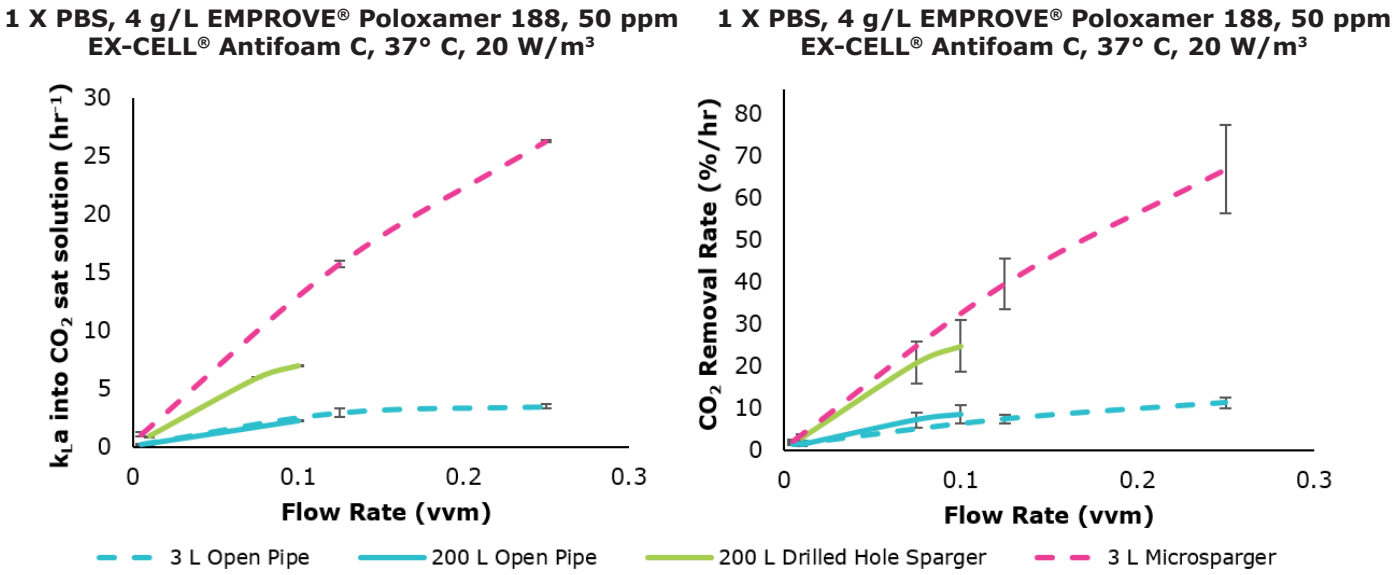


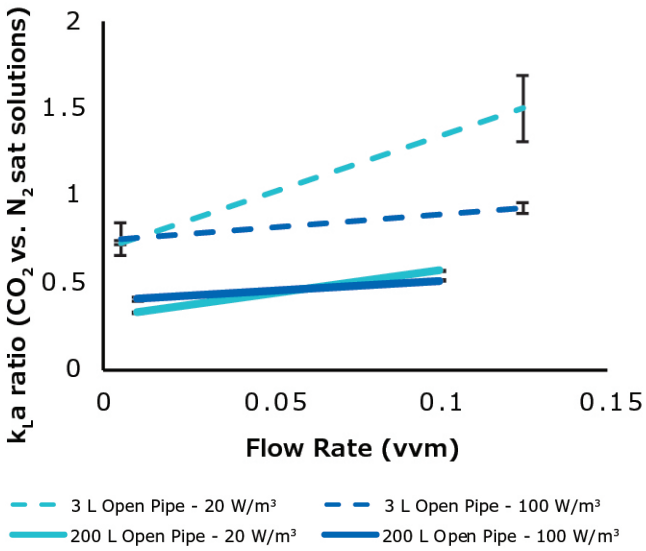
Figure 12. CO_2 stripping endpoint results from 3 L to 200 L scale. 3 L data is the average ± SD of n=2 Mobius® 3 L Single-use Bioreactors and the 200 L data is the average ± SD of two CO_2 or DO probes run n=2 times per condition.

3 L and 200 L k_La ratio in mock media solutions

To understand why CO_2 accumulates faster at larger scales, CO_2 stripping k_La (CO_2 saturated solution) was compared to oxygenation k_La (N_2 saturated solution) within the same mock media solution. Findings suggests that for the open pipe, scaling by oxygenation k_La results in decreased CO_2 stripping at larger scale.

Alternatively, the drilled-hole and microsparger showed consistent CO_2 stripping efficiency independent of scale. While the k_La ratio shows that the open pipe is more efficient for CO_2 stripping than oxygenation, the drilled-hole/microsparger had higher overall CO_2 stripping capabilities and was more consistent when scaling by oxygenation k_La.

1 X PBS, 4 g/L EMPROVE® Poloxamer 188, 50 ppm EX-CELL® Antifoam C, 37° C



1 X PBS, 4 g/L EMPROVE® Poloxamer 188, 50 ppm EX-CELL® Antifoam C, 37° C

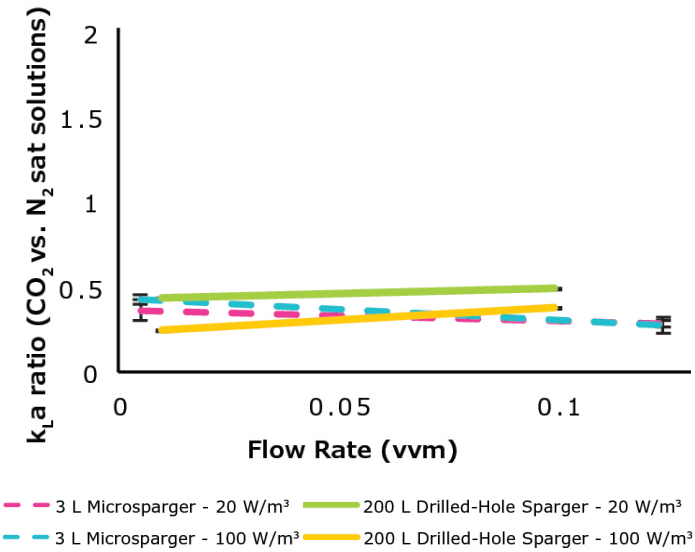


Figure 13. k_La ratio of CO_2 stripping vs. oxygenation (N_2) for open pipe spargers and drilled hole/microsparger at bench and pilot scale. Values greater than one indicate a higher k_La into CO_2 saturated solution than N_2.

Summary

Key engineering parameters were characterized to compare design spaces for the Mobius® 3 L Single-Use Bioreactor and the Mobius® 50-2000 L iFlex Bioreactors to provide the toolkit for developing cell culture processes that can achieve consistent performance across scales. A power density of >90 W/m³, an impeller tip speed of <2.2 m/s, a <33 second mixing time, and a <700 s^{-1} estimated shear rate can be achieved at full volume across the entire range of vessel sizes from the Mobius® 3 L Single-Use Bioreactor to the Mobius® 50-2000 L iFlex Bioreactors. Additionally, a k_La >50 hr^{-1} can be achieved on both Mobius® 3 L Single-Use Bioreactor and Mobius® 200 L iFlex Bioreactor at 2 L and 200 L working volume, respectively. Bubble size delivered by all included sparger designs were characterized to help understand bubble shear risks and develop more effective gassing strategies when scaling from the Mobius® 3 L Single-Use Bioreactor to the Mobius® iFlex Bioreactors. Lastly, CO_2 stripping efficiency was assessed to understand trends per sparger type during scale-up.

Equations

		Equation	Variables
Eq. 1	Power Density	$P_d = P/V$	P = power V = volume
Eq. 2	Angular Velocity	$\omega = RPM_{avg} * (1\ min)/(60\ sec)$	RPM = revolutions per minute
Eq. 3	Power	$P = \tau * \omega$	τ = torque ω = angular velocity
Eq. 4	Power Number	$N_P = P/(\rho d^5 \omega^3)$	ρ = density of mixing fluid d = impeller diameter
Eq. 5	Reynold's Number	$Re = (\omega d^2 \rho)/\mu$	μ = viscosity of mixing fluid
Eq. 6	Tip Speed	$v_{tip} = \omega * d * \pi$	d = impeller diameter
Eq. 7	Tip Shear Rate	$\gamma_{tip} = 2\pi DN/H$	D = impeller diameter H = impeller height N = rotational velocity
Eq. 8	Cup Shear Rate	$\gamma_{cup} = (2\pi R_i N)/(R_o - R_i)$	R_i = inner cup radius R_o =outer cup radius
Eq. 9	Gas Entrance Velocity	Flow Rate/(Open Area (Number of Holes * Hole Area))	
Eq. 10	Bubble Diameter	$D = \sqrt{}$(min f eret * max f eret)	min f eret = minimum bubble diameter max f eret = maximum bubble diameter
Eq. 11	Mass Transfer Mass Balance at Steady State Conditions	$OTR = k_L a(C^* - C)$ $OUR = VCD * qO_2$ $OTR = OUR$	OTR = oxygen transfer rate OUR = oxygen uptake rate VCD = viable cell density qO_2 = cell specific oxygen uptake rate k_La = volumetric mass transfer rate
Eq. 12	Mass transfer coefficient (k_La)	$k_L a = OTR/(C^* - C)$	C^* = saturation concentration C = oxygen concentration C_1 = initial gas concentration
Eq. 13	Mass transfer coefficient (k_La)	$\ln (C^* - C_1)/(C^* - C) = k_L a * (t - t_1)$	t = time, t_1 = initial time

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3. Chisti, Yusuf. "Animal-cell damage in sparged bioreactors." Trends in biotechnology 18, no. 10 (2000): 420-432.
4. Chaudhary, Garima, Robin Luo, Meena George, Lia Tescione, Anurag Khetan, and Henry Lin. "Understanding the effect of high gas entrance velocity on Chinese hamster ovary (CHO) cell culture performance and its implications on bioreactor scale-up and sparger design." Biotechnology and bioengineering 117, no. 6 (2020): 1684-1695.

Related Documents

- Scalability and performance guide PG12163EN
- BPI Impeller poster PS9878EN
- BPI Sparger poster PS9879EN
- Mobius® iFlex Bioreactor Datasheet DS12340EN
- Mobius® 3L Bioreactor Specifications SP2345000

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