Scalability of Mobius® Single-Use Bioreactors

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Introduction

With the increased adoption of single-use bioreactor platforms in upstream biomanufacturing processes, there is a need to expand single-use offerings to include larger production equipment to complement existing smaller bench and pilot scales systems. In order to successfully implement a large scale single-use bioreactor platform, its ability to obtain equivalent performance (scalability, with smaller sized bioreactors across the platform) must be considered during its design and ultimately demonstrated.

One approach used for successful scale-up of a biomanufacturing process across a bioreactor platform is to maintain geometric similarity between the vessels, such as tank aspect ratio, impeller placement and design, sparger type and location as well as the inclusion of an appropriate baffle system. However, it is not always possible to maintain the same key bioreactor parameters such as shear, mixing time and kLa identical between small and large vessels. Therefore, successful scale-up of a biomanufacturing process also must consider the effects of critical process parameters such as gas flow rates and impeller agitation speed on the cell culture environment across the multi-volumetric bioreactor systems. We have therefore developed and characterized a family of single-use bioreactors, for mammalian cell growth and recombinant protein production, including bench-scale (3 L), small-scale (50 L), pilot-scale (200 L), and clinical-scale (1000 L and 2000 L) bioreactor spanning early process development through clinical batch production. The performance design space of the entire Mobius® Bioreactor platform was characterized using several key engineering parameters including oxygen mass transfer coefficient (kLa), power per unit volume, Reynolds number (Re), mixing time and tip speed. Based on a detailed understanding of the dynamic performance capabilities of each bioreactor system across the platform, appropriate process parameters can be selected to achieve scalable performance.

3 L 50 L 200 L 1000 L 2000 L

Parameter	3 L	50 L	200 L	1000 L	2000 L
Working Volume: Total Vol.	0.8	0.8	0.8	0.8	0.8
Impeller Diameter: Vessel Diameter	0.6	0.3	0.3	0.3	0.3
Vessel Height: Vessel Diameter	1.8:1	2.0:1	2.0:1	2.0:1	2.0:1
Liquid Height: Vessel Diameter	1.4:1	1.7:1	1.6:1	1.6:1	1.6:1
Internal Baffle	N/A	Single (Paddle-type)		Single (X-type)	
Impeller Power Number (Np)	0.3	3.2	4.0	3.5	3.3
Min – Max Working Volume	1 – 2.4 (L)	10 – 50 (L)	40 – 200 (L)	200 – 1000 (L)	400 – 2000 (L)
Microsparger	Sintered polyethylene	Membrane polyethylene			
Open Pipe Sparger	2.3 mm	1.5 mm		4.0 mm	
Impeller Design	Up-pumping 3 blade marine; centered on shaft	Up-pumping 4 blade pitched blade; bottom mounted 15° off center			
Vessel Heating	Electric heating blanket	Stainless steel liquid jacket			
Probes Ports	3 top plate probe ports	Mobius® SensorReady technology (up to 8 ports)			
Probe Type	Fits standard 12 mm PG 13.5 threaded probes				

Mixing and Power Input

Mixing is a critical bioreactor performance characteristic responsible for minimizing gradients and maintaining control within the cell culture environment. Good mixing should evenly distribute bioreactor contents, helping to minimize zones of uneven cell density, pH, temperature, dissolved gases and nutrient or waste concentrations, while minimizing the shear stress imparted on the cells by the fluid dynamics or the mixing element itself. Baffle design was considered during the development of the Mobius® Single-use Bioreactors to promote efficient mixing across scales.

Figure 1: Agitation Effects Summary

Reynolds number, impeller tip speed and impeller RPM correlating to a 1, 10 and 20 W/m^3 power input in each of the Mobius® Bioreactor vessels summarizes capabilities across scales.

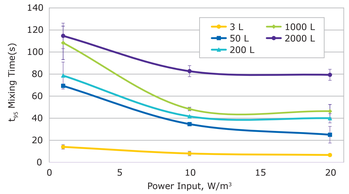
Bioreactor	W/m^3	RPM	tip speed (m/s)	Re
3 L	1	82	0.3	8,817
	10	178	0.7	19,140
	20	224	0.9	24,086
50 L	1	61	0.3	13,288
	10	131	0.7	28,537
	20	165	0.9	35,943
200 L	1	38	0.4	23,535
	10	81	0.8	50,168
	20	102	1.0	63,174
1000 L	1	33	0.5	48,097
	10	72	1.1	104,940
	20	90	1.3	131,174
2000 L	1	32	0.6	64,612
	10	69	1.2	139,319
	20	87	1.5	175,663

Figure 2: Colorimetric Mixing Quality

Colorimetric mixing studies performed in a prototype 2,000 L scale tank show excellent homogeneous mixing quality. Note, the flexible film baffle is present and critical during these studies.

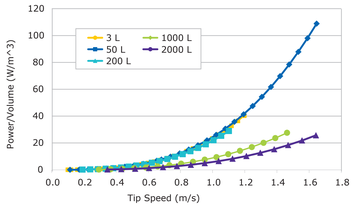
0 sec 10 sec 20 sec 30 sec 40 sec 50 sec

Figure 3: Mixing time vs. Power Input



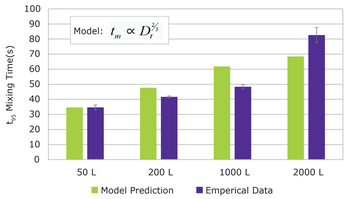
Figures 3 maps the conductivity profiles from probes placed in the Mobius® SensorReady loop or in the top plate port in the case of the 3 L. t95 is the time when the profile reached 95% of its final value. A salt solution was added at the liquid surface to induce a 25 – 50 mS increase. Triplicates were performed at each test point.

Figure 4: Power Input vs. Tip Speed



Figures 4 outlines each bioreactor's relationship between impeller tip speed and power per unit volume.

Figure 5: Empirical Mixing data vs. Scaling Model



Figures 5 demonstrates that at the same P/V (10 W/m^3) the mixing time in the Mobius® 3 L, 50 L, 200 L 1000 L and 2000 L bioreactors scales comparably to the predictive model for increased mixing time with increased vessel diameter.

Cell Culture Performance

The following data highlights that from the bench top (3 L) to clinical processing (2000 L) cell culture performance in Mobius® Bioreactors is scalable. In this study, a Mobius® 2000 L Single-use Bioreactor was inoculated with CHO cells for an 11 day trial. A 3 L Mobius® Single-use Bioreactor was inoculated from the same culture source and run in parallel. The data are compared to a historical Mobius® 200 L Single-use bioreactor trial with the same process. Initial results show that cell density, viability and productivity are equivalent across the scales.

Figure 6: Viable Cell Density and Viability Profiles

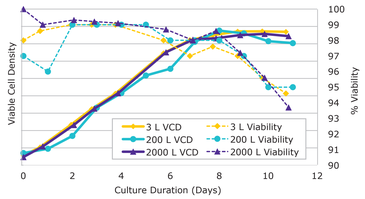
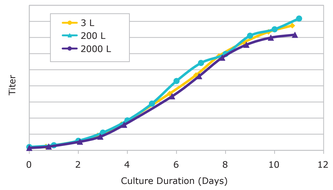


Figure 7: Product Titer Profiles



Mass Transfer of Gas (k_La)

One of the most critical performance parameters for bioreactors is mass transfer of gasses. To assess the gas transfer efficiency of the Mobius® bioreactor process containers, the volumetric mass-transfer coefficients (k_La) for oxygen were measured using the static gassing out method with PBS and a power input of 10W/m^3. The uniform DO interval used for the calculation was between 10% and 90% air saturation for triplicate trials.

Figure 8: Scalable Mass Transfer Summary

Bioreactor	Air Flow Rate (vvm)	Microsparger k_La (hr -1)	Air Flow Rate (vvm)	Open Pipe k_La (hr -1)
3 L	0.5	23	0.1	3
50 L	0.04	22	0.05	3
200 L	0.03	23	0.03	3
1000 L	0.03	22	0.03	3
2000 L	0.05	22	0.02	3

Summary

Detailed characterization of engineering parameters in addition to vessel geometries were studied during design and development of the Mobius® bioreactors to support a mechanistic approach to achieving scalable performance across the wide range of process volumes.

From bench scale (3 L) through Clinical Scale (2,000 L) the family of Mobius® Single-use Bioreactors are capable of delivering comparable cell culture performance enabling successful scale up and scale down of biomanufacturing processes.

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