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Application Note

Scalability of the Mobius® Single-use Bioreactors

Abstract

The life science business of Merck has developed a family of single-use bioreactors designed for mammalian cell growth and recombinant protein production. The Mobius® Single-use Bioreactor product offering includes bench-scale (3 L), pilot-scale (50 L and 200 L), and clinical-scale (1000 L and 2000 L) bioreactors spanning early process development through clinical batch production. To enable the successful scale-up of a biomanufacturing process, a number of factors critical to efficient cell growth, viability and protein production were considered and used to optimize the design of the bioreactors. These include mixing efficiency, gas transfer capability, and an operating range of power input that minimizes cell shear. In this study, several key design parameters, such as the volumetric mass transfer coefficient for oxygen (k_La), power per unit volume, Reynolds number (Re), mixing time and tip speed were characterized for the five different sized single-use bioreactors. Based on these data, CHO cells were cultured in these bioreactors maintaining equivalent power per unit volume as the primary scaling parameter. These data provide evidence of cell culture scalability across the entire Mobius® Single-use Bioreactor platform.

Introduction

The Mobius® Bioreactor family includes the bench-scale (3 L), pilot-scale (50 L and 200 L), and clinical-scale (1000 L and 2000 L) bioreactors that enable cell culturing capabilities for early process development through clinical batch production. The bench-scale Mobius® 3 L Bioreactor is a rigid stirred tank bioreactor (Figure 1A) while the Mobius® 50 L, 200 L, 1000 L and 2000 L large-scale bioreactors (Figures 1B, 1C, 1D and 1E) are inflatable stirred tank Flexware® assemblies used in stainless steel vessels. Table 1 outlines the features of all five bioreactors. For all sizes greater than bench-scale, the Mobius® Bioreactors utilize the novel Mobius® SensorReady Technology for process monitoring and control. The SensorReady assembly is an external loop that is connected to the Flexware® assembly which allows for a configurable number of probes to be used.

The ability to scale-up a biomanufacturing process is essential for process development and the production of biotherapeutic proteins. Bioreactor process set points, acceptable ranges and general operating parameters used at the large scale are commonly based on those developed at the bench top or small

Figure 1A.
Mobius® 3 L Bioreactor

Figure 1B.
Mobius® 50 L Bioreactor

Figure 1C.
Mobius® 200 L Bioreactor

Figure 1D.
Mobius® 1000 L Bioreactor

Figure 1E.
Mobius® 2000 L Bioreactor

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Table 1. Design Characteristics of Mobius® Bioreactors

Parameters	3 L	50 L	200 L	1000 L	2000 L
Total Volume (L)	3	60	250	1250	2500
Minimum Working Volume (L)	1	10	40	200	400
Maximum Working Volume (L)	2.4	50	200	1000	2000
Total Height to Diameter Ratio (nominal)	1.8:1	2.0:1	2.0:1	2.0:1	2.0:1
Liquid Height to Diameter Ratio (at max working volume)	1.4:1	1.7:1	1.6:1	1.6:1	1.6:1
Vessel Diameter (cm)	13.7	34	54	92	116
Impeller Diameter (cm)	7.6	10.9	18.3	27.9	33.0
Impeller Geometry	Up-pumping marine (3 blades)	Up-pumping pitched blade (4 blades at 13° pitch)			
Impeller Position	On shaft, centered	Bottom mount, 15° from center			
Impeller Power Number (Np)	0.3	3.2	4.0	3.5	3.3
Baffles	NA	Single (Paddle-Type)		Single (X-Type)	
Open Pipe Sparger Orifice	2.3 mm	1.5 mm		4.0 mm	
Microsparger Type	Sintered polyethylene	Membrane polyethylene			
Vessel Heating	Electric heating blanket	Stainless steel liquid jacket			
Control	3 probe ports	Mobius® SensorReady Technology			
	Fits standard 12 mm PG 13.5 threaded probes				

scale where experimentation is more cost effective and efficient. Additionally, large scale performance investigations and production expectations are often greatly dependent upon results obtained at smaller scales. It is therefore important that the small scale bioreactors effectively model larger scale systems and vice versa.

Scaling up bioreactor processes is challenging; even with similarly designed tanks, it is difficult to simultaneously maintain equivalent key bioreactor characteristics such as hydrodynamic shear and mixing time.[1] Other variables, such as bubble size and distribution, nutrient regulation and delivery, and process control capabilities may also contribute to variable performance results across scales. Ultimately, successful scale-up is determined when comparable process performance endpoints such as cell growth, cell viability, protein production (i.e., titer) and product quality are all achieved. The probability of meeting these criteria can be increased when the bioreactor systems are well-characterized and the process design space is well understood.

In this study, several key parameters including oxygen mass transfer capability (k_La), power per unit volume, Reynolds number, mixing time and tip speed were characterized for the five Mobius® Single-use Bioreactors. CHO cells were then cultured in these bioreactors based on maintaining an equivalent power per unit volume as the primary scaling parameter. The results of these studies define the characterization and process design space offered by the bioreactors and demonstrate the capability to achieve expected cell culture performance across scales, thus demonstrating the scalability of the family of Mobius® Single-use Bioreactors.

Results

Scaling Techniques

Although no single parameter can guarantee comparable process performance between stirred tank bioreactor systems, choosing an agitation rate that delivers equivalent energy dissipation rate or power per unit volume (W/m^3) is a common first approach.[5,6] The impeller design, fluid density and agitation rate are considered in the ungassed power per unit volume (P_o/V) equation:

$$\frac{P_o}{V} = N_P \cdot \rho \cdot N^3 \cdot D^5$$

Where
P_o = Ungassed power input
V = Liquid working volume
N_p = Impeller power number
ρ = Fluid density
N = Impeller agitation rate
D = Impeller diameter

Using this scaling method, agitation rate is varied to maintain similar ungassed power per unit volume across vessels.

The highest shear zones in a stirred tank bioreactor are often described as existing within the impeller zone. Because the outer edge of the impeller blades create shear as they rotate through the fluid, the impeller tip speed is often considered during bioreactor comparisons:

$$\text{Tip Speed} = \pi \cdot D \cdot N$$

Where
D = Impeller diameter
N = Impeller agitation rate

The impeller tip speed calculation, however, focuses on a local condition and takes neither fluid flow conditions nor impeller geometry or design into consideration.

The Reynolds number can be considered when estimating fluid conditions at a given agitation rate by providing a ratio of the inertial forces to the viscous forces:

$$Re = \frac{\rho N D^2}{\mu}$$

Where
ρ = Fluid density
N = Impeller agitation Rate
D = Impeller diameter
μ = Fluid dynamic viscosity

The system is considered fully turbulent when Re > 10,000.[2,3] The Reynolds number calculation (in water at 25 °C) is based on the assumption of a cylindrical tank design with a centered rotating impeller and does not take impeller design into account. The Mobius® large scale bioreactors contain a bottom mounted, offset, pitched blade impeller and a baffle which together increase the turbulence and improve mixing.

Mobius® 2000 L Bioreactor

Table 2 outlines the impeller power number, tip speed, Reynolds number and ungassed power per unit volume values calculated for a range of applicable agitation rates for the Mobius® 3 L, 50 L, 200 L, 1000 L and 2000 L Bioreactors.

Table 2. Agitation Effects Summary

Bioreactor	W/m^3	RPM	Tip Speed (m/s)	Re
3 L (N_p = 0.3)	1	82	0.3	8,890
	10	178	0.7	19,297
	20	224	0.9	24,284
50 L (N_p = 3.2)	1	61	0.3	13,397
	10	131	0.7	28,771
	20	165	0.9	36,238
200 L (N_p = 4.0)	1	38	0.4	23,728
	10	81	0.8	50,579
	20	102	1.0	63,692
1000 L (N_p = 3.5)	1	33	0.5	48,097
	10	72	1.1	104,940
	20	90	1.3	131,174
2000 L (N_p = 3.3)	1	33	0.6	67,177
	10	70	1.2	142,497
	20	87	1.5	177,104

Mixing

Mixing is a critical bioreactor performance characteristic because it is responsible for minimizing gradients and maintaining control within the cell culture environment. Good mixing in a bioreactor strives for sufficient fluid pumping and turnover throughout the system to effectively create a single homogeneous environment which can be accurately monitored and controlled. Good mixing should evenly distribute bioreactor contents, helping to minimize zones of uneven cell density, pH, temperature, dissolved gasses and nutrient or waste concentrations, while minimizing the shear stress imparted on the cells by the fluid dynamics or the mixing element itself.

Mixing time was evaluated for the large scale Mobius® Bioreactors by observing conductivity probe response curves measured from a probe located in the SensorReady assembly. A salt solution was introduced at the liquid surface, and the mixing time was determined as the time when the conductivity profile had reached 95% of its final value (t_{95}). Each trial was performed in triplicate and results are shown in Figure 2. The Mobius® 3 L Bioreactor system was evaluated in a similar manner with the conductivity probe inserted in the head plate probe port.

The results in Figure 2 show that at equivalent power per unit volume, the average mixing time in the Mobius® Bioreactors increases with vessel size. This is to be expected as larger liquid volumes result in longer fluid paths. In order to achieve a well-mixed condition in the same amount of time, the fluid velocity would have to increase for the condition in the larger volume tank. As a guide, fluid velocity is proportional to the square root of power per unit volume in turbulent conditions (i.e., when Re > 10,000).[3,4] As a result, an increase in power per unit volume would be required to achieve similar mixing times in larger vessel volumes. For scaling from one vessel to another size vessel with similar geometry and with the same power per unit volume in turbulent conditions, the mixing times are related by the following equation:[7]

$$\frac{t_{T2}}{t_{T1}} = \left(\frac{D_2}{D_1}\right)^{11/18}$$

Where t_{T1} = Mixing time in tank 1
t_{T2} = Mixing time in the tank 2
D_1 = Impeller diameter in tank 1
D_2 = Impeller diameter in tank 2

The Flexware® assemblies for the Mobius® 50 – 2000 L Bioreactors contain a single baffle, which coupled with the off-center, angled position of the bottom mounted impeller, provide good mixing dynamics even at lower power inputs. The similar geometry of these four bioreactors allows for application of the model.

Mixing times in the Mobius® 200 L, 1000 L and 2000 L Bioreactors were predicted based on the 50 L mixing data as a reference point. The results in Figure 3 show the mixing times as predicted from this model compared to the empirical mixing data generated in each system at 10 W/m³. The correlation between the model prediction and testing results demonstrates that scalable mixing efficiency is achieved across all scales. Please refer to the Mobius® Single-use Bioreactors Performance Guide for more details on mixing characterization.

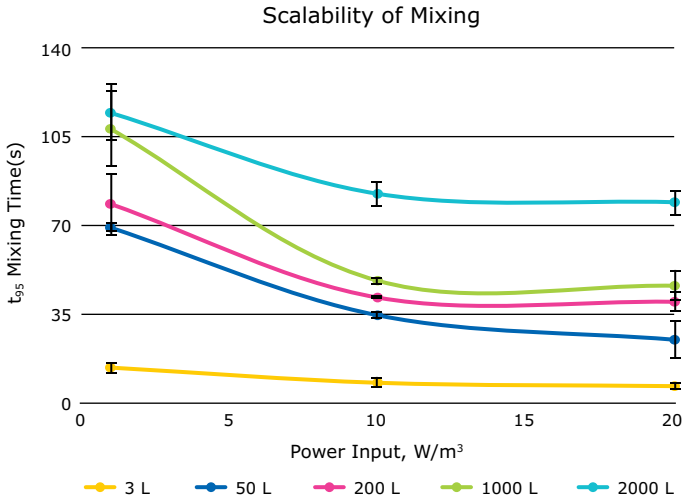

Figure 2.
Average Mixing Times of the Family of Mobius® Bioreactors. Data points represent the average mixing time of n=3 individual trials. Error bars represent one standard deviation for each data point.

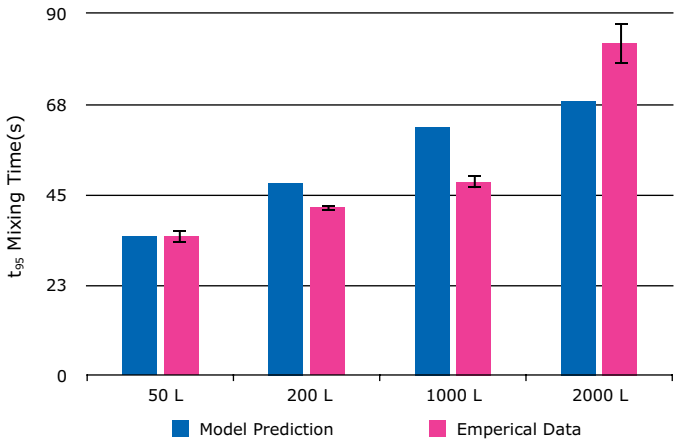

Figure 3.
Average Mixing Time vs. Theoritical Model. Emperical data are compared to the theoretical model prediction at a power per unit volume input of 10 W/m³.

Efficient Gas Transfer

One of the critical scale-up parameters used for bioreactor control is the mass transfer of gasses. Sufficient O_2/Air delivery is required not only to support cell growth, metabolism and protein production but also to control CO_2 accumulation in the media which can impact both of these critical performance endpoints.[4] To assess the gas transfer efficiency of the Mobius® Bioreactors, the volumetric mass-transfer coefficients (k_La) for oxygen were measured in each bioreactor using the static gassing out method. For these trials, equal power per unit volume (10 W/m^3) was used as the scaling parameter for impeller speed and gas flow rates within the operating window for each system were chosen to achieve equivalent gas transfer efficiency.

k_La values were determined by filling the bioreactors to the volumes listed in Table 3 with a phosphate buffered saline solution and setting the temperature to 37 °C. The dissolved oxygen was stripped from the bioreactors by supplying nitrogen gas via the microsparger. Once the DO concentration was less than 2% air saturation, the nitrogen supply was turned off and air was introduced into the bioreactor through the microsparger or the open pipe sparger at the flow rates indicated in Table 3. The DO concentration was recorded until its saturation was achieved. An air overlay was not used in these studies.

k_La Calculation: The k_La value for each trial was calculated from the DO vs. time graph. To avoid subjectivity in determining the k_La, the calculation was based on DO concentrations between 10% and 90% of the measured air saturation. The k_La values shown represent the slope of the line created by plotting the following equation versus time (t_2-t_1):

$$\ln\left(\frac{C^* - C_{t1}}{C^* - C_{t2}}\right)$$

Where C^* = DO saturation concentration
C_{t1} = Initial DO concentration
C_{t2} = DO concentration at time 2
t_1 = Initial time
t_2 = Time 2

As detailed in Table 1, the impeller and sparger design and placement in the Mobius® 50 L, 200 L, 1000 L and 2000 L Bioreactors are all similar. However, the design used in the Mobius® 3 L Bioreactor is different. For example, in the Flexware® assemblies for the large-scale Mobius® Bioreactors the membrane polyethylene microsparger is located directly beneath the impeller, while in the 3 L Bioreactor a sintered polyethylene microsparger is located off to the side of the impeller. Also, the impeller blade shape differs between the bioreactors. A pitched blade impeller is used in the Flexware® assemblies for the large scale Mobius® Bioreactors whereas a marine impeller is found in the 3 L Bioreactor. Despite these differences, it is possible to demonstrate scalable k_La values between the five bioreactors operating at constant power per unit volume by adjusting the air flow rates used. As shown in Table 3, the k_La values measured in the five bioreactors using the microsparger and a power per unit volume of 10 W/m^3 are comparable. Likewise similar, and hence scalable k_La values were obtained with the open pipe spargers using this same approach. By leveraging such an approach, k_La scalability can be developed for a variety of power inputs and operating volumes across the platform.

Table 3. Scalable Gas Transfer

Mobius® Bioreactor	Working Volume	Microsparger Air Flow Rate (vvm)	Microsparger k_La (hr^{-1})	Open Pipe Air Flow Rate (vvm)	Open Pipe k_La (hr^{-1})
3 L	2 L	0.50	23	0.10	3
50 L	50 L	0.04	22	0.05	3
200 L	200 L	0.03	23	0.03	3
1000 L	1000 L	0.03	22	0.03	3
2000 L	2000 L	0.05	22	0.02	3

*Vessel volumes per minute (vvm) = air flow rate/operating volume

Cell Culture

Maintaining a homogenous environment within the bioreactor is the most critical criterion for a successful cell culture process. While different agitation strategies may be used to scale-up a biomanufacturing process, power per unit volume is the most commonly used scaling parameter.[5,6] The second most important criterion for a successful cell culture run is effective mass transfer of gasses to maintain cell growth, productivity, and product quality expectations. Using these criteria, CHO cell culture batch processes were performed in the Mobius® 3 L, 50 L, 200 L and 2000 L Bioreactors using power per unit volume as the primary scaling parameter to demonstrate cell culture scalability across the family of Mobius® bioreactors. Gas flow rates were chosen to achieve similar k_La values for each vessel.

A CHO-S (CHO-S 14C4 mAb04) cell culture batch process was performed utilizing the Mobius® 3 L, 50 L, and 200 L Bioreactors. The process parameters for each scale are outlined in Table 4.

Table 4. Operating Parameters for CHO-S Cell Cultures

Operating Parameters	Mobius® 3 L Bioreactor	Mobius® 50 L Bioreactor	Mobius® 200 L Bioreactor
Agitation Rate	200 rpm (14 W/m³)	147 rpm (14 W/m³)	91 rpm (14 W/m³)
Air Overlay	None	0.01 vvm	0.01 vvm
Dissolved Oxygen	30% (Air sparge on/ off, 0.1 vvm)	30% (Air/O_2 continuous PID, 0.02 vvm)	
pH	6.95 ± 0.5 with CO_2 and 0.5M Na_2CO_3		
Temperature	37 °C		

To demonstrate scalable cell culture performance, parameters including cell growth, viability and metabolism were compared. For this study, samples were analyzed daily on a Vi-Cell® XR (Beckman Coulter), a BIOPROFILE® FLEX system (NOVA Biomedical) and a Blood Gas Analyzer (Siemens Rapidlab® 248). As shown in Figure 4A, the viable cell densities and viabilities were comparable between the Mobius® 3 L, 50 L and 200 L Bioreactors. As shown in Figure 4B and 4C, the nutrient profiles and metabolic rates between the three scales were also comparable.

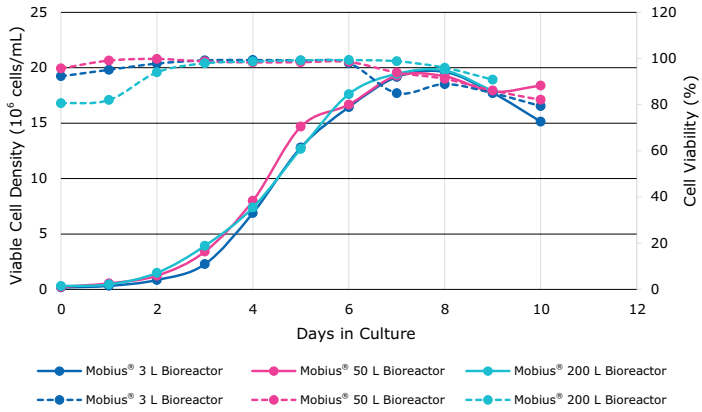

Figure 4A.
Viable Cell Density (solid lines) and Viability (dashed lines) vs. Culture Time for a Batch Culture Process at the 2 L, 50 L and 200 L Working Volumes. Data was obtained daily from the Vi-Cell® XR (Beckman Coulter).

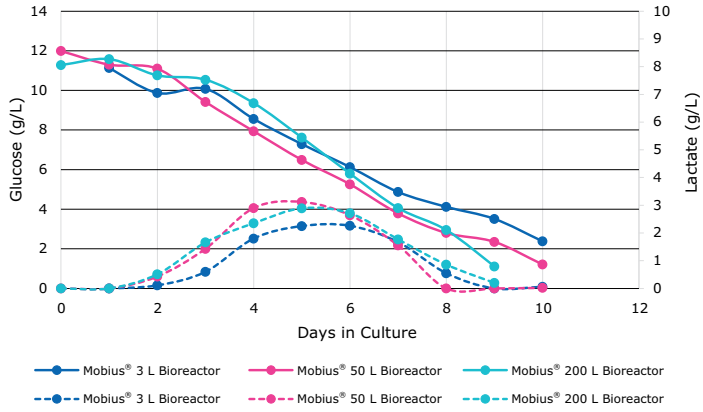

Figure 4B.
Glucose (solid lines) and Lactate (dashed lines) Concentrations vs. Culture Time for a Batch Culture Process at the 2 L, 50 L and 200 L Working Volumes. Data was obtained daily from the BIOPROFILE® FLEX system (NOVA Biomedical).

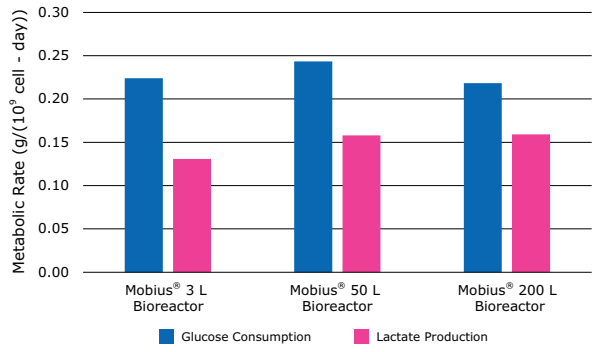

Figure 4C.
Metabolic Rates for Culture Days 1-5 for a Batch Culture Process at the 2 L, 50 L and 200 L working volumes. Data was obtained daily from the BIOPROFILE® FLEX system (NOVA Biomedical).

A second mAb-producing CHO-S cell line (S148/2120014C1) was similarly evaluated for performance across the Mobius® Single-use Bioreactor platform using process parameters that had been developed specifically for this cell line. This study included independent cell culture runs conducted in the Mobius® 50 L, 200 L and 2000 L Bioreactors with the Mobius® 3 L Bioreactor data generated in parallel to the 2000 L bioreactor run. The data from these single-use bioreactors were compared to historical data (n = 10) generated over four years of experience with this cell line using a 1250 L stainless steel bioreactor. In all cases, the cell viability profiles were similar throughout the batch culture runs (Figure 5A). The cell growth profiles obtained using the four single-use bioreactors were similar to one another and to that obtained with the 1250 L stainless steel bioreactor. The variability inherent to this legacy process is evidenced by the error bars associated with the historical stainless steel data set. Additionally, recombinant mAb protein production was observed to be similar between the different single-use bioreactors and compared well to that obtained using the 1250 L stainless steel bioreactor (Figure 5B).

The comparable cell growth, viabilities, metabolic profiles and protein titers obtained with these two independent cell lines demonstrate that scalable performance both between the Mobius® Bioreactors themselves and versus stainless steel systems can be achieved. This is facilitated by the design of the Mobius® Bioreactors, understanding system performance characterization of key engineering parameters, as well as by appropriately applying scaling rules during process development.

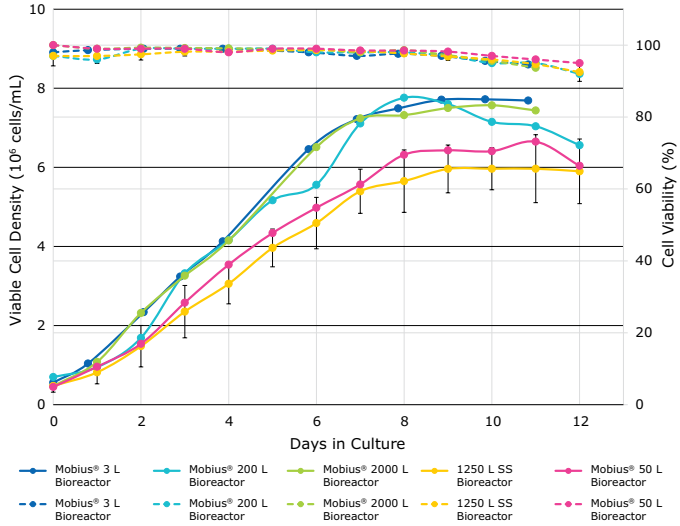

Figure 5A.
Viable cell density (solid lines) and viability (dashed lines) vs. culture time for a batch culture process in the Mobius® 3 L, 50 L, 200 L and 2000 L Single-use Bioreactors as Compared to a 1250 L Stainless Steel Bioreactor. The data were obtained daily from a Vi-Cell® XR (Beckman Coulter) analyzer.

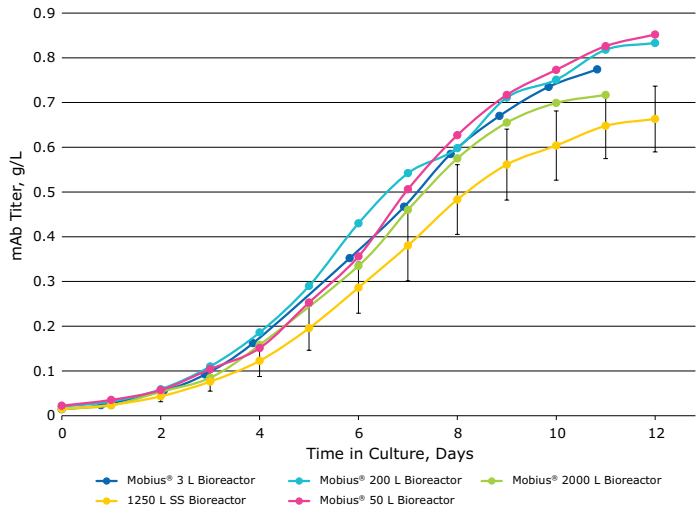

Figure 5B.
Monoclonal Antibody Production during a batch culture process in the Mobius® 3 L, 50 L, 200 L and 2000 L Bioreactors versus a 1250 L stainless steel bioreactor. Samples were taken daily from each bioreactor and analyzed for protein titer.

Conclusions

Successful bioreactor scaling is dependent on several factors inherent to the system design. For the Mobius® Single-use Bioreactor family, vessel geometry, impeller power number, and routine calculations of power input, tip speed and Reynolds number are provided to establish a baseline for building a scalability method. The mixing time for each vessel was studied and summarized in comparison to a predictive model of mixing time to show that the Mobius® Bioreactor family has been designed to achieve scalable mixing performance. Similarly, equivalent k_La values are experimentally shown to be achieved by tuning the gas flow rate at equivalent power inputs for comparability across all five vessel sizes for both the microsparger and the open pipe sparger.

Through this series of calculations and experiments highlighting several key engineering parameters generally known to be effective during process scaling, it has been shown that the Mobius® Single-use Bioreactor family is capable of delivering a scalable process design space from bench scale, through clinical production scale. For a true demonstration of this scaling capability, two independent cell culture processes were studied to examine cell culture process performance as demonstrated by cell growth, viability, metabolism and product titer. Not only does the cell culture data support the scalability within the Mobius® Single-use Bioreactor family it also provides evidence that the Mobius® Bioreactors are scalable to stainless steel bioreactors by applying the same traditional bioreactor scaling methods.

In summary, the family of Mobius® Single-use Bioreactors has been designed with scalability as a critical performance criteria. They meet the scalability expectations based on engineering principles and models and can be successfully scaled to achieve comparable process performance with each other as well as with traditional bioreactors typically used in biomanufacturing of mammalian cell culture processes.

References

1. Shuler, M., and Kargi, F. (2002). Bioprocess Engineering Basic Concepts, 2nd ed., Prentice Hall, Inc.
2. Dalian University of Technology. Mixing, Chapter 6. http://ceb.dlut.edu.cn/uploads/soft/110415/7-110415154330.pdf
3. Nienow, A.W. (2006). Reactor engineering in large scale animal cell culture. *Cytotechnology* **50**:9-33
4. Marks, D.M. (2003). Equipment design considerations for large scale cell culture. *Cytotechnology* **42**:21-33
5. Xing, Z. (2009). Scale-up Analysis of a CHO Cell Culture Process in Large-Scale Bioreactors. *Biotechnology and Bioengineering* **103**:733-746.
6. Kaiser, S.D. et al, (2011) CFD for Characterizing Standard and Single-use Stirred Cell Culture Bioreactors. In *Computational Fluid Dynamics Technologies and Applications*, InTech.
7. Geankoplis, C.J. (2003). Transport Processes and Separation Process Principles, 4th ed., Pearson Education, Inc. Publishing as Prentice Hall Processional Technical Reference

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