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Technical Brief

Mobius® Single-use Bioreactors: Platform Scalability

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Adoption of single-use bioreactor platforms across all scales, ranging from benchtop to production, are becoming increasingly popular due to their ease of use and operational flexibility. Knowledge of the performance characteristics at small-scale allows predictable and easy scale-up across the platforms from 3 L to 2000 L bioreactors.

We have developed and characterized the Mobius® family of single-use bioreactors for mammalian cell culture and recombinant protein production ranging from bench scale (3 L) to small scale (50 L), pilot scale/early clinical (200 L) and late clinical/ commercial scale (1000 L and 2000 L) (Figure 1).

Figure 1: Mobius® single-use bioreactors 3 L, 50 L, 200 L, 1000 L and 2000 L

Bioreactor processes are generally developed and characterized at small scale prior to scale-up. This detailed understanding of the dynamic performance capabilities of each system across the platform allows selection of process parameters to achieve scalable performance with a high degree of confidence.

Bioreactor processes have scale independent parameters such as pH setpoint, DO setpoint, temperature, feed volume ratios, inoculation density, nutrient concentrations, as well as scale dependent parameters that can be adjusted to maintain process similarity with increasing bioreactor scale. 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 (P/V), mixing time and tip speed.

The successful scale-up of an upstream biomanufacturing process 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. 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.

Scale-up of a bioreactor manufacturing process

We provide clear characterization of key engineering parameters, including power input per volume, mixing time, oxygen transfer capabilities and temperature mapping across all scales of the Mobius® bioreactor portfolio. Thorough characterization of these key parameters, coupled with consistent geometric scaling ratios, provides confidence that the Mobius® bioreactors will meet process needs for adherent cultures, when using microcarriers, or suspension cell cultures.

Scale dependent parameters include the size of the bioreactor, how the contents of the vessel are added and mixed, and how the mixture is oxygenated. These parameters include:

- Geometrical similarity between the bioreactors in the family: **vessel height/diameter ratio** (usually a 2:1 aspect ratio for Mobius® family), is held constant.
- **Impeller design** for determining the speed and efficiency of mixing. Impellers can be a scoping marine or pitched blade impeller with 3- or 4-blades mounted on a centered shaft or they can be bottom mounted and agitate through a spinning magnet or magnetic field coupled to the impeller. Baffles can be added to the vessel to break up the vortex and improve mixing efficiency through turbulent mixing.
- **Tip speed** is a rudimentary form of scale-up since it doesn't take impeller and bioreactor design into account. This is typically used when no other information about the impeller is available.
- **Power input per volume (P/V)** normalizes the impeller designs and agitation rates to the volume in the bioreactor. The power per unit volume is calculated by using the power number of the impeller (a dimensionless number), density of the fluid, the rotational speed and the diameter of the impeller and the volume of liquid in the bioreactor.
- **Mixing time** is an important scaling factor as mixing is critical to ensure that when nutrients are added, the culture achieves fast homogeneity that eliminates gradient formation of the nutrients. Mixing is also important to eliminate low oxygen or nutrient dead zones within the culture during the process. Mixing is scalable with a knowledge of the power input (W/m^3) for each bioreactor size. Mixing is also dependent upon the presence and design of baffles.
- **Oxygen mass transfer coefficient (kLa):** The mass transfer characteristics of a system that determine the ability to provide oxygen to cells. The kLa is typically determined experimentally by using the gassing out method in a mock media and is expressed in inverse hours.

Bioreactor design considerations for scale-up

Mixing is a critical bioreactor performance characteristic responsible for minimizing gradients and maintaining control within the cell culture environment. A bottom-mounted impeller maintains gentle agitation during the clarification step down to the minimum working volume without generating foam and delivering a consistent harvest. 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.

In the absence of a baffle, an impeller can result in the formation of a vortex during mixing. The presence of a vortex, especially at larger scales, can result in zones that are not completely mixed. To overcome this issue, the Mobius® bioreactor benefits from an internal baffle resulting in homogeneous mixing in under a minute at low power input.

Several sparger options are available to provide process flexibility. Microspargers or ring spargers, also called drilled hole spargers (DHS), are typically used for the aeration of the culture and located directly under the impeller to promote the dispersion of bubbles and provide maximum mass transfer of oxygen (kLa). While the microspargers have the smallest bubble size and achieve the highest kLa, there can be issues with cells that are sensitive to bubble shear. Microspargers also produce the highest amount of foam, so foam management is critical when using a microsparger. Although ring spargers have a larger bubble size than the microsparger and a lower kLa, they produce less bubble shear and much less foam. The type of aeration sparger is highly dependent on the oxygen requirements of the culture.

Open pipe spargers are another type of sparger, typically located adjacent to the impeller and are efficient at delivering macro bubbles. These spargers produce a much larger bubble and thus a much lower kLa and are inefficient for delivering oxygen to typical cell density cultures but are efficient at stripping CO_2 from liquid. Therefore, open pipe spargers are mostly used to strip accumulated CO_2 from the culture to reduce the amount of base delivered during the cell culture process.

The oxygen mass transfer coefficient (kLa) is one of the most critical performance parameters for bioreactors and represents the mass transfer of gases between the gas and liquid phases. This is a function of agitation/mixing and sparge rates. To assess the gas transfer efficiency of the Mobius® bioreactor process containers, the volumetric mass-transfer coefficients (kLa) for oxygen were measured using the static gassing out method with PBS and a range of power per unit volume and air flow rates. The kLa value was determined from the most linear portion of the oxygen accumulation curve for triplicate trials.

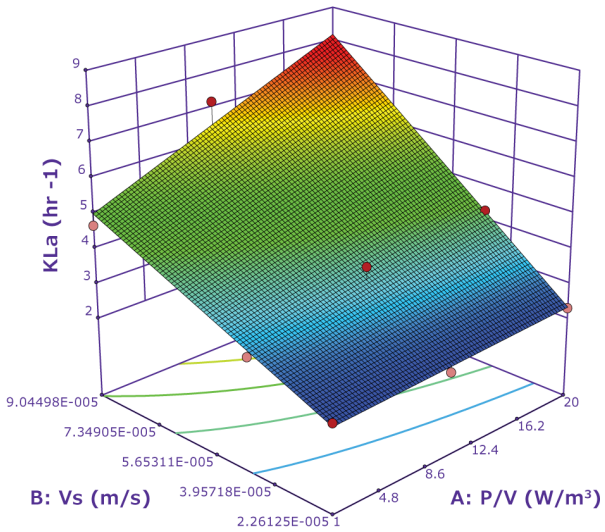
Putting the data together

Model development:

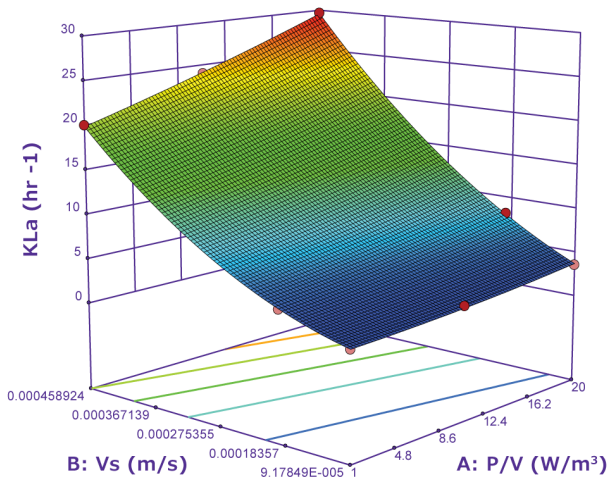
Mass transfer of gas (kLa) was measured across the range of bioreactor sizes. Holding the sparge rates constant at a given volume and power/unit volume (W/m3) allows the modeling of system characteristics. Mass transfer modeling shows that kLa is a function of power/unit volume (P/V (W/m3)) and the superficial gas velocity (Vs (m/s)).

kLa = f (P/V; Vs)

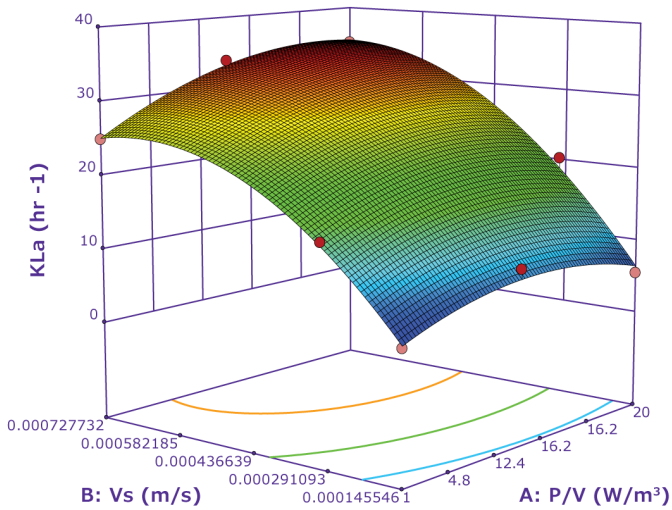
Three factor plots were constructed showing kLa (hr-1) vs A: P/V (W/m^3) and B: Vs (m/s) for each bioreactor system data set (Figure 2).



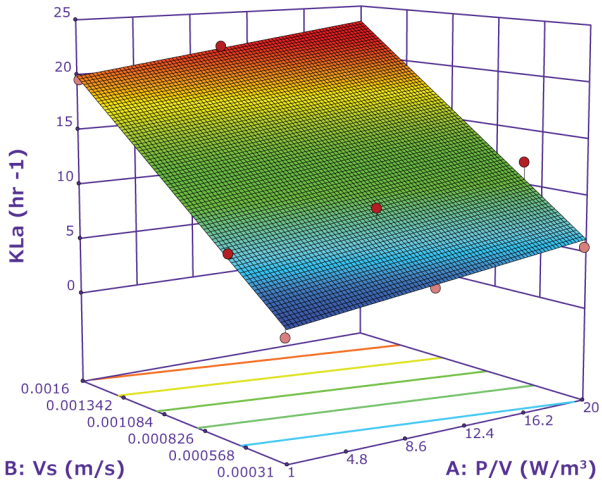
Mobius® 3 L bioreactor



Mobius® 50 L bioreactor



Mobius® 200 L bioreactor



Mobius® 2000 L bioreactor

Figure 2: Three factor plots were constructed showing kLa (hr-1) vs A: P/V (W/m3) and B: Vs (m/s) for each bioreactor system data set

Cell culture performance:

Using the combination of the equipment knowledge and the mass transfer modeling, scale-up consistency was assessed. A scale-up case study was performed using bioreactors from 3 L to 2000 L. Cell cultures were carried out for 16 days in 3 L, 200 L and 2000 L. The agitation rate and air flow through the microsparger at the different scales were chosen to maintain the same kLa values across the scales. For all three bioreactor sizes, the viable cell density curves were comparable along with the peak viable cell density of ~40 x 10^6 cells /ml (Figure 3) at day 8-9. The viability curve (Figure 4) was also comparable at each scale staying within the range of 90-100% through each process. The metabolic parameters for pH (Figure 5) and lactate (Figure 6) were similar across all three bioreactor sizes. The titer curves for each scale were also comparable (Figure 7). This indicates that a knowledge of the equipment and technology transfer leads to scale-up success.

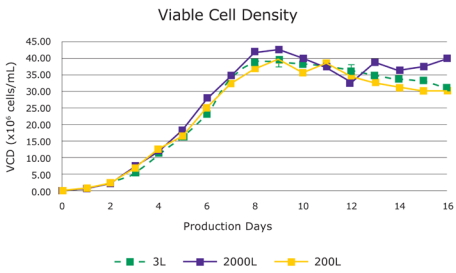


Figure 3. Comparison of the viable cell density curves of the three bioreactor sizes

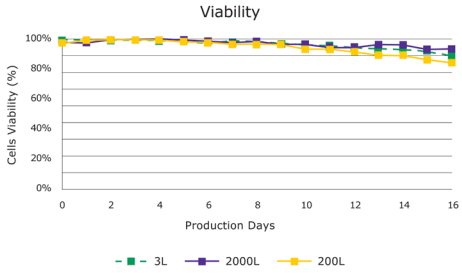


Figure 4. Comparison of the three bioreactor sizes viability curve

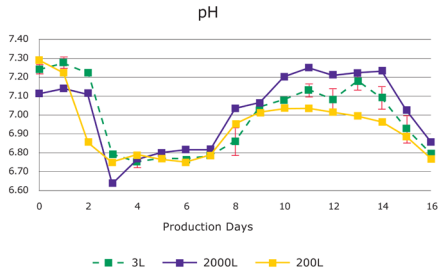


Figure 5. Metabolic parameters for pH of the three bioreactor sizes

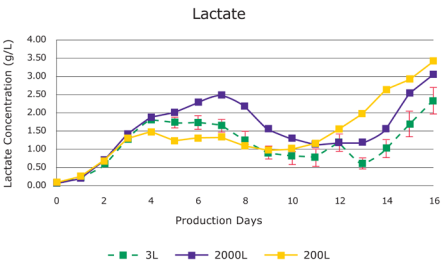


Figure 6. Metabolic parameters for lactate of the three bioreactor sizes

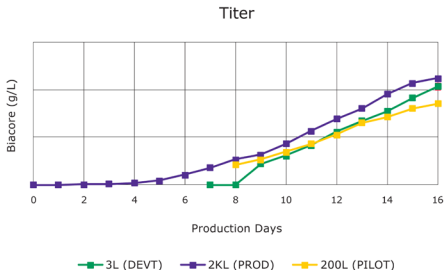


Figure 7. Comparison of titers for the three bioreactor sizes

Conclusion:

Characterization of the engineering parameters during the design and development of the Mobius® bioreactors support a mechanistic approach to scalable performance from the bench top to production scale process. The family of Mobius® bioreactors from 3 L through 2000 L can deliver comparable cell culture performance allowing successful scale-up and scale down of biomanufacturing processes.

Acknowledgement:

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