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Technical Brief

Mixing and Gas Transfer in the Mobius® Breez Microbioreactor – How It Works?

A 2 mL automated single-use perfusion cell culture platform, the Mobius® Breez Microbioreactor, enables increased efficiency and reduced development timelines over traditional small-scale models, such as 96-deep well plates, spin tubes, and bench-scale bioreactors. The platform is comprised of gamma-irradiated microbioreactor cassettes with integrated fluid supply, four bioreactor controllers (PODs), utility hardware, and control software.

Four bioreactors can operate simultaneously, each with its own independent POD controller that provides closed loop control for pH, dissolved oxygen (DO), temperature, and cell density via optical density (OD) sensors, giving you automated throughput at milliliter-scale. With a novel mixing mechanism, mixing and oxygenation supply is enabled through inflation and deflation of a gas permeable silicone membrane within the microbioreactor cassette layers. Fast mixing is possible without high shear, resulting in high cell viability during culture.

This technical brief describes the unique mixing and gas system of the Mobius® Breez Microbioreactor, which provides advantages over classical cell culture research tools by enabling well-controlled conditions for proper process development and optimization in perfusion applications.

Mixer Design and Operation

The Mobius® Breez cassette design incorporates microfluidic input and output lines, a rigid chamber engineered for cell culturing, control instrumentation such as valves and pumps, optical sensors, and a polyethersulfone (PES) perfusion filter. The growth chamber consists of three sections that have connectors on the edges to form a contiguous ring (Figure 1). Each section can be independently filled or emptied utilizing gas pressure and holds approximately 1 mL when full. Additionally, the growth chamber connectors pull vacuum continuously to remove unwanted bubbles from the cell culture. During normal operation, two chamber sections are full, and one section is empty, resulting in a working volume of 2 mL.

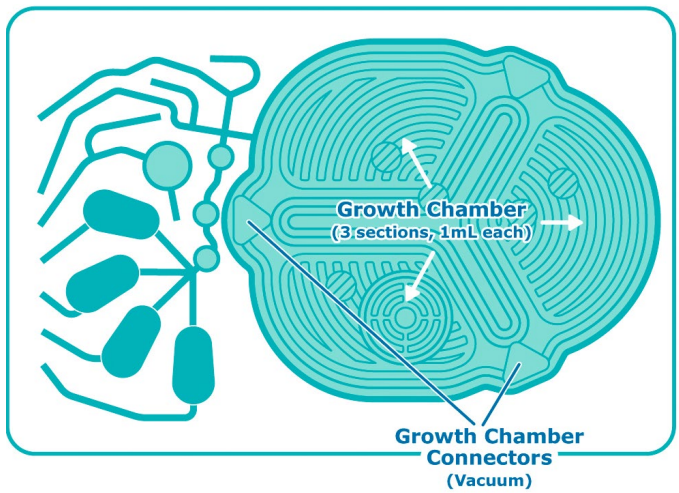

Figure 1.

Mobius® Breez cassette with the three sections and connections of the growth chamber indicated.

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A silicone membrane separates each chamber section into a top side for gas and a bottom side for liquid. To mix, the silicone membranes are inflated sequentially from the air side, which pushes the liquid out of one section into the neighboring sections (Figure 2). The gas used to drive mixing also provides gas transfer. As the silicone membrane enables rapid gas diffusion, mixing mediates O_2 and CO_2 exchange between the air and liquid sides.

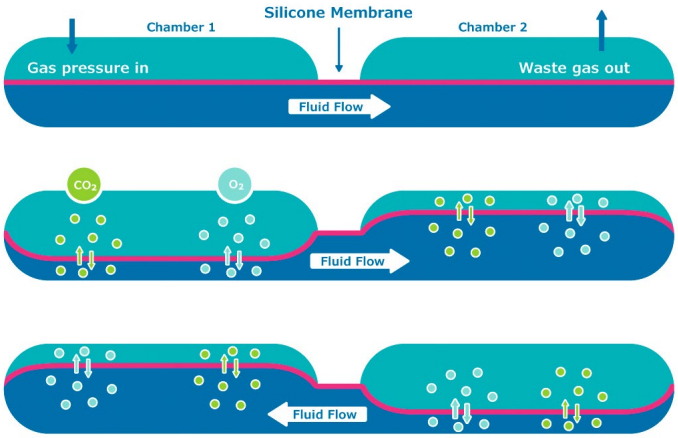


Figure 2.

Cross-sectional view of two connected growth chamber sections, illustrating how movements of the silicone membrane mediate fluid flow and gas exchange.

Gas Exchange

The Mobius® Breez Microbioreactor achieves gas transfer rates with kLa (volumetric mass transfer coefficient) of 30–40 h^{-1}. Unlike stirred tank bioreactors, gas transfer for all gases occurs through the same liquid-gas interface via the silicone membrane, so gas transfer rates of O_2 and CO_2 are similar. In addition, gas delivery and mixing are coupled since the gas used to inflate the silicone membranes is also the gas that diffuses into the liquid. This means that the headspace gas flowrate is very high compared to a benchtop stirred tank (e.g., 3 L). As a point of reference, the standard gas flowrate through the headspace of the microbioreactor is approximately 0.2 L/min, or 100 VVM (vessel volume per minute), compared to typical gas flow rates in stirred tank bioreactors of <0.1 VVM.

The boundary condition of the liquid-gas interface remains fixed at the concentrations of the source gas entering the headspace due to high gas flow rates. For CO_2 levels during a cell culture experiment, this boundary condition means that it is critical to maintain a minimum level of CO_2 in the headspace, with a recommended value equal to the CO_2% set for incubator culture.

Without a minimum CO_2% at low cell densities, the pH controller can reduce the CO_2% to zero to counteract acid accumulation and cause cell growth to slow or stop due to low dissolved CO_2 (dCO_2) in the cell culture. Figure 3 shows an example of how cells contribute to the total dCO_2 in the reactor at different CO_2 kLa values under a fixed headspace gas concentration boundary condition.

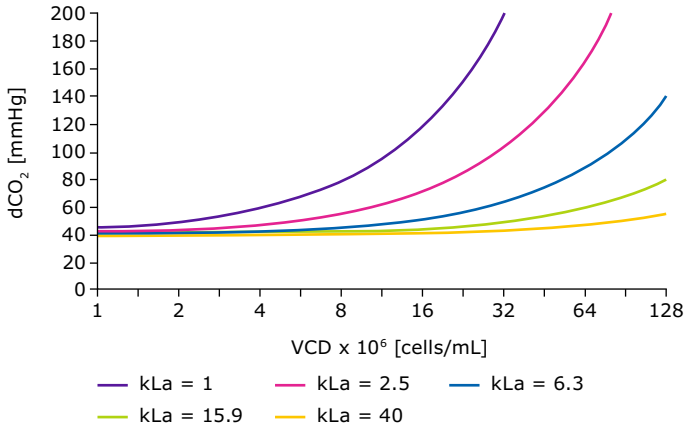


Figure 3.

dCO_2 as a function of cell density at different CO_2 kLa values. A headspace overlay of 38 mmHg (5%), qCO_2 = 4 pmol/cell/day and steady state is assumed.

Mixing Controls

The high gas transfer rates achieved can have a side effect of bubble generation when the gas consumption rate of the liquid is low (e.g., when the cell density is low). While the vacuum connectors help with bubble removal, the surface area of the pressure sections is higher than the vacuum sections, therefore mixing controls are also employed to reduce gas delivery into the liquid.

Mixing is controlled by three software parameters: mixing frequency, intermittent delay, and mixing cycles per intermittent delay. Default settings for mixing operation in the Mobius® Breez Microbioreactor are based on testing CHO cell aggregation, growth, and viability under varying mixing conditions (data not shown).

Mixer Frequency

The mixing frequency is related to time spent sending gas into a section to inflate one silicone membrane. Typically, the mixer is set to 5 Hz, which equates to 200 ms gas flow per section. Since there are three sections, a full mixing cycle (Figure 4) is three times the mixer frequency. In the default state, each section would be pressurized for 200 ms sequentially, taking a total of 600 ms to complete one cycle.

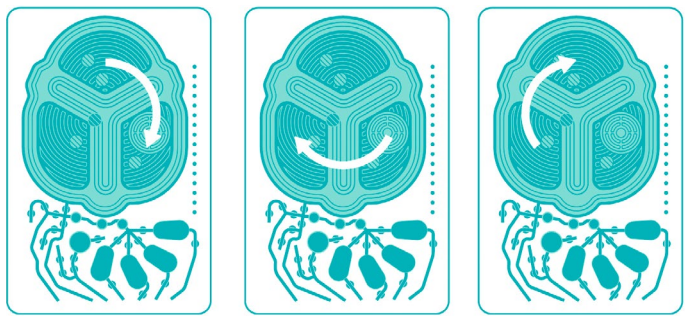


Figure 4.

Circulation of liquid in a full mixing cycle.

For a given mixer frequency, the amount of movement of the silicone membrane is illustrated in Figure 5. Lower mixer frequencies correspond to longer inflation times per section, resulting in more deflection of the silicone membrane and more fluid movement per stroke.

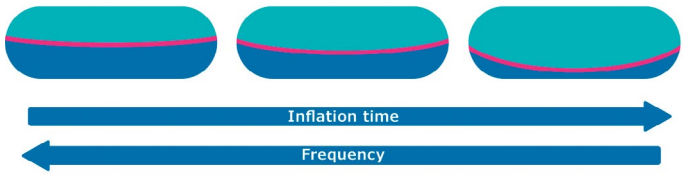


Figure 5.

Relative changes in membrane deflection with changing inflation time.

Intermittent Delay

Intermittent delay is the duration of time when mixing is off during each period of the mixing cycle. No movement of the membranes occurs for this duration. If this parameter were to be too long, cells would have an opportunity to settle, therefore the system is configured to avoid this undesirable condition.

By default, intermittent delay is configured to the maximum of 16.35 seconds at the start of an experiment. It automatically reduces to 0 seconds (continuous mixing) when the system detects oxygen is being consumed by cells and additional oxygen is needed by the cells. However, it can be manually held to a fixed value by the operator to account for situations like high inoculation density and detecting changes in cell-specific oxygen consumption rates. It is recommended to not change the default value without consulting our technical experts.

Mixing Cycles per Intermittent Delay

Mixing cycles per intermittent delay is a parameter that specifies the duration when mixing is on. A higher number means more mixing is performed before the next intermittent delay starts. A lower number means fewer mixing cycles are performed. Therefore, the combination of mixing cycles and delay determines one full period (Figure 6).

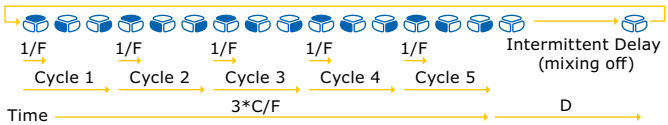


Figure 6.

Overview of full mixing period. The mixer frequency is denoted as F, the intermittent delay duration as D, and the number of cycles as C.

The full mixing period as given in Figure 6 can be written as:

Period = 3 x C/F + D

When solving for the ratio of mixing time versus the full period, we arrive at the equation below. This equation is plotted in Figure 7 with a frequency of 5 Hz and 5 cycles per mixing delay, which are the software default values.

% time spent mixing = 3×C/3×C+D×F

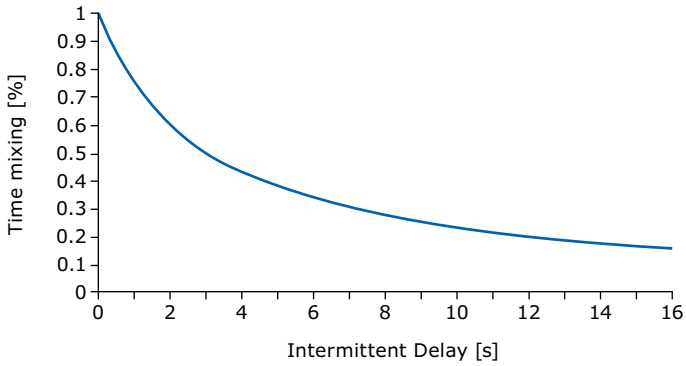


Figure 7.

Amount of mixing per mixer program period for a given delay. Frequency = 5 Hz and Cycles = 5.

Customized Mixing Control

A physical flow restrictor inside the Mobius® Breez POD impacts the speed that the gas in a mixing section can exhaust. This restrictor is a global control over the speed that each membrane can deflect and send gas out the vent when the chamber is filling with liquid. This parameter is preset to an optimal value and is not adjustable by default. Adjustment of the physical flow restrictor is not recommended without technical support.

Process Effects

Low Shear Stress

Due to a distinct configuration and mixing strategy, the gassing and mixing principles of the Mobius® Breez Microbioreactor are different than those of stirred tank bioreactors. Stirred tank bioreactors utilize blade impellers to create a high-power mixing environment, while the microbioreactor utilizes a flexible silicone membrane that creates a wave-like motion and results in shear stress at the low range of stirred tank bioreactors (see related resources for more information on microbioreactor shear). Furthermore, the silicone membrane has a large surface area with high permeability, which allows rapid gas exchange while not generating bubbles in the microbioreactor culture chamber. A comparison of gassing and agitation parameters between a 3 L glass bioreactor and the microbioreactor is summarized in Table 1.

Parameter	Microbioreactor	Glass Bioreactor
Impeller/Agitator	Flexible silicone membrane	Dual marine-style blade
Estimated shear	>1 Pa	>1 Pa
Mixing frequency	5 Hz	350–400 rpm
Shear protectant	Poloxamer 188	Poloxamer 188
Sparger	N/A	L-sparger & microsparger
Bubble	No bubbles	Yes, varying sizes
Antifoam	N/A	Up to 0.4%
CO_2%	6%	6%

Table 1.

Gassing and agitation comparison between the Mobius® Breez Microbioreactor and a 3 L glass bioreactor.

High Oxygen Transfer Rate

In stirred tank bioreactors, oxygen molecules are transported from gas bubbles (gas phase) to the media (liquid phase). The gas exchange efficiency is a function of the bubble size and quantity. The 3 L glass bioreactor for example, which uses an L-sparger to aerate the culture, relies heavily on the bubble formation to efficiently oxygenate the media. Higher kLa values were obtained at higher mass flow rate and higher agitation rate. However, even at the maximum mass flow and agitation rates, only ~20 h^{-1} kLa was obtained with the 3 L glass bioreactor. In the Mobius® Breez Microbioreactor, gases are directly transferred to the liquid phase through the actuating membrane, which has a large surface area, allowing the microbioreactor to have higher kLa values compared to the glass bioreactor (Figure 8).

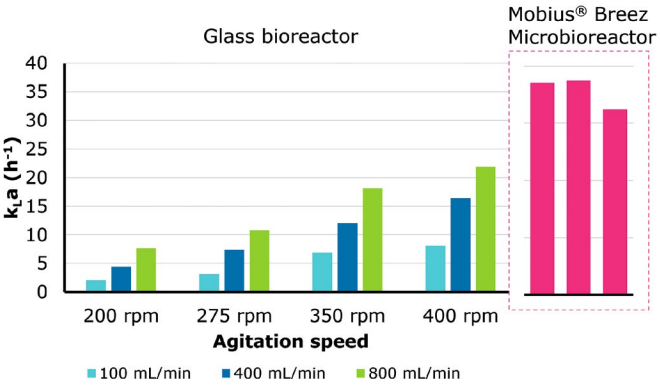


Figure 8.

Gas transfer comparison showing the difference in kLa between a 3 L glass bioreactor and the Mobius® Breez Microbioreactor.

Yet, Predictive of Bench-Scale Bioreactors

We hypothesized that the differences in the gassing and agitation strategies between the microbioreactor and the glass reactor would not affect cellular viability. Perfusion cultures on multiple cell lines were performed using both types of bioreactors accounting for a wide range of CHO families (Figure 9). Viability values were selected at a low CSPR range (<30 pL/c/d). All experiments were set at 40% DO level. Cells cultured in both systems showed comparable viabilities.

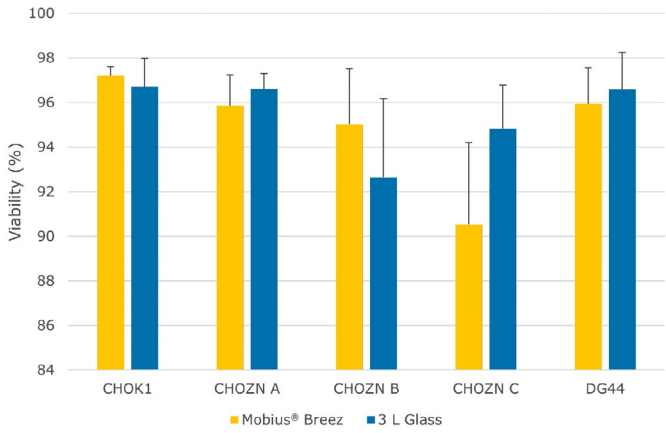


Figure 9.

Viability of multiple CHO cell lines cultured either in the 2 mL microbioreactor or 3 L glass bioreactor using EX-CELL® Advanced HD perfusion medium.

Next, we evaluated the effect on cellular productivity by comparing the Mobius® Breez Microbioreactor to various stirred tank reactors using different cell lines and media. Three CHOZN® GS and one CHOK1 clones were cultured in microbioreactors and in either 1, 3, or 15 L bench-scale glass bioreactors. Catalog culture media was either EX-CELL® Advanced HD perfusion medium or Cellvento® 4CHO-X medium. Cellular productivities were plotted against CSPR (Figure 10). All the CHOZN® clones show comparable productivity between the Mobius® Breez Microbioreactor and glass bioreactors. CHOK1 produced slightly less in the glass compared to the microbioreactor.

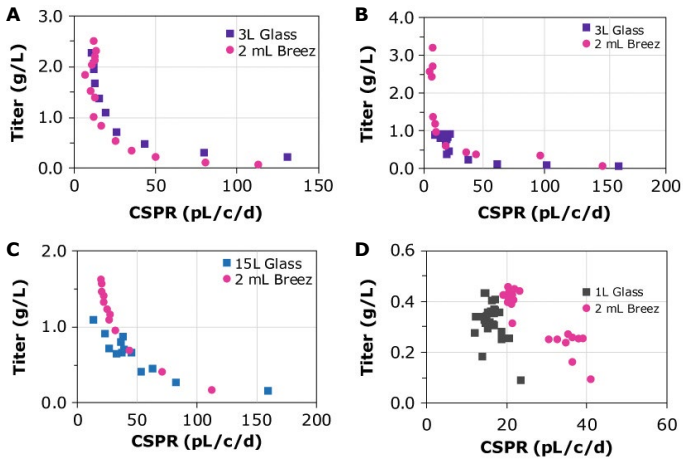


Figure 10.

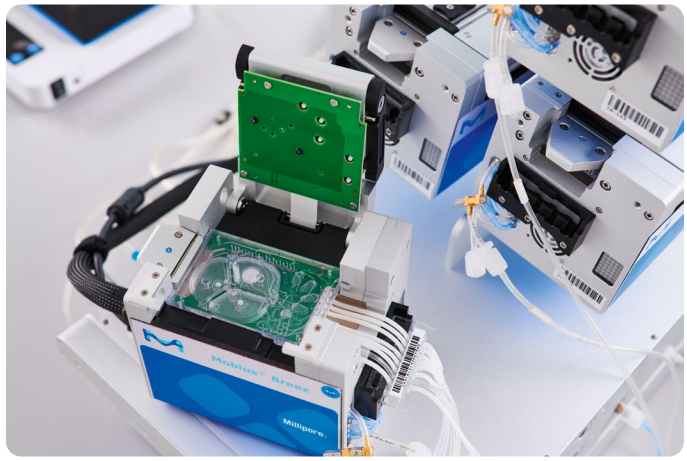
Titer vs CSPR comparison using the 2 mL microbioreactor and 1, 3, or 15 L glass bioreactors on various CHOZN® GS clones cultured in EX-CELL® Advanced HD perfusion medium (A and C) or Cellvento® 4CHO-X medium (B) and CHO-K1 cultured in EX-CELL® Advanced HD perfusion medium (D).

Conclusion

The Mobius® Breez Microbioreactor implements an innovative design that maximizes mixing and delivers ideal gas exchange. Compared to stirred tank reactors, variations such as shear environment, bubble formation, and gas transfer mechanism are evident. However, while the mixing and gassing strategy for the microbioreactor is distinct, the 2 mL bioreactor shows to be a suitable small-scale tool for stirred tank bioreactors in terms of predicting trends for productivity and cell growth. Early discovery and development studies with the Mobius® Breez Microbioreactor offer scientists the ability to predict perfusion cell culture performance before investing time and costs in larger scale experiments. The small volume and automated perfusion of the Mobius® Breez Microbioreactor provides complete control and flexibility to optimize cell culture media, screen for cell line clones, and perform early-stage process development for perfusion applications.

Related Resources

1. Schwarz, H., Lee, K., Castan, A., & Chotteau, V. (2023). Optimization of medium with perfusion microbioreactors for high density CHO cell cultures at very low renewal rate aided by design of experiments. Biotechnology and Bioengineering, 120, 2523–2541. https://doi.org/10.1002/bit.28397
2. Webinar (2023). A single-use, scale down, predictive solution for intensified perfusion development.



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