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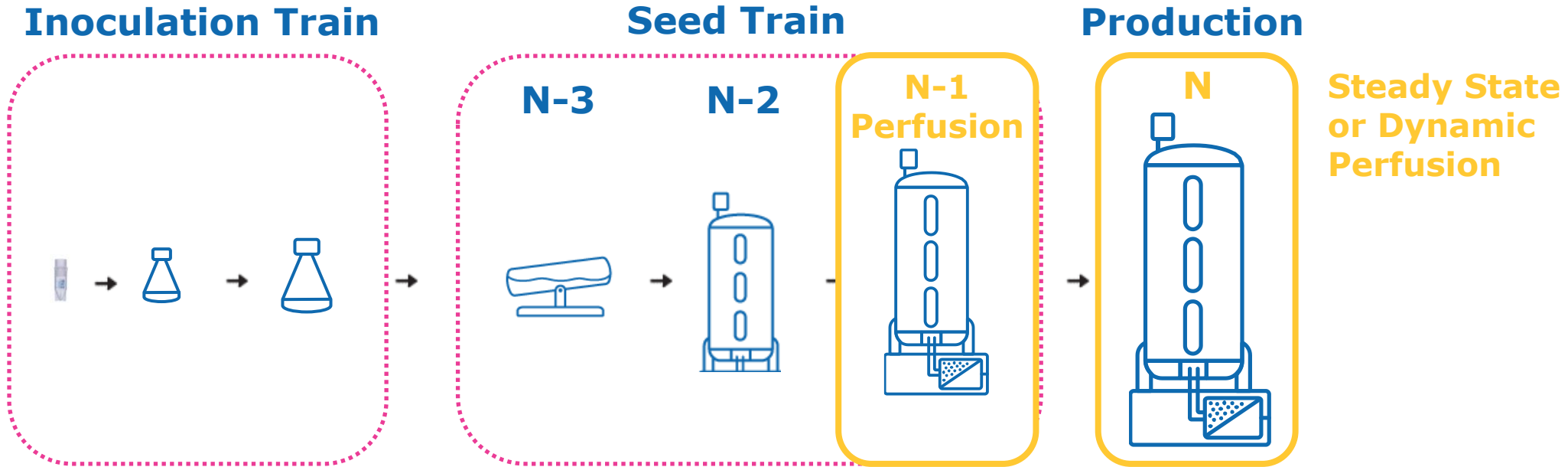
Preparation, Separation,
Filtration & Monitoring Products

Optimizing Cell Retention and Oxygenation for a High-Performance Perfusion Bioreactor Platform

Allyson Caron
June 20th, 2024

**MILLIPORE
SIGMA**

Upstream process intensification strategies rely on perfusion to improve process economics and yield



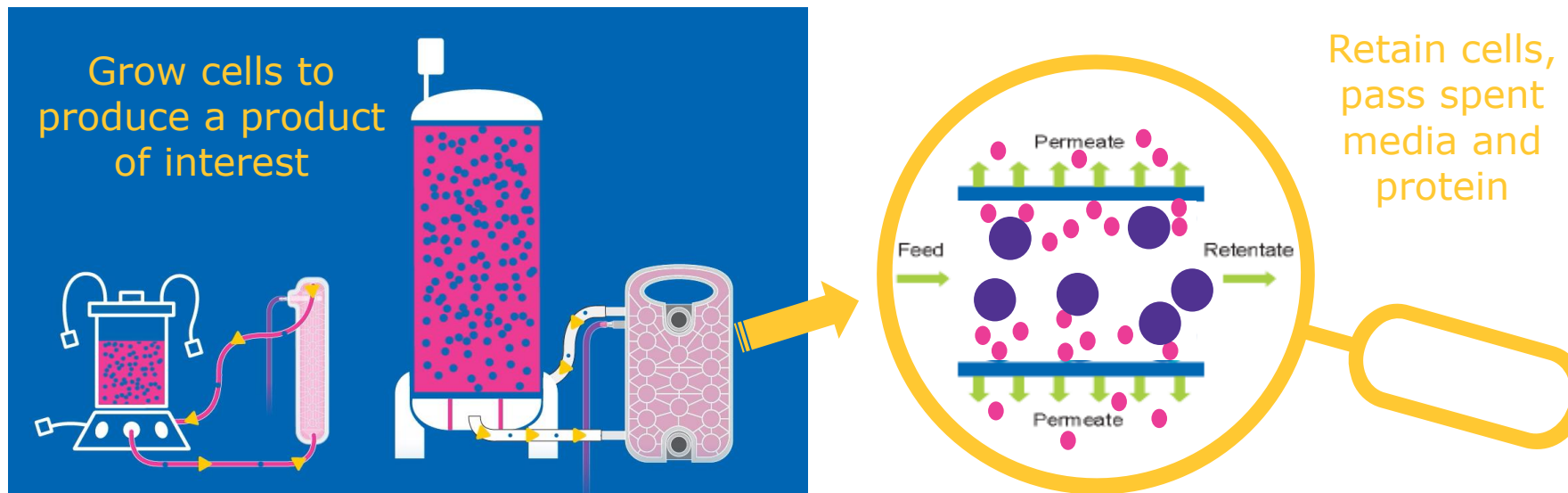
Intensified Seed Train

- Achieves higher cell densities
- Allows for seeding multiple bioreactors, larger bioreactors, or high seed bioreactors
 - Saves process time
 - Reduces manufacturing footprint

Intensified Production

- Enables longer process durations at higher cell densities than fed-batch
 - Allows for continuous processing
 - Increases productivity

Realization of perfusion benefits requires addressing common technology challenges



Common Bioreactor Challenges:

- Ineffective or non-optimized oxygenation strategy
- Unexpected challenges due to poor scalability
- Bag leaks
- Complexity with process control
- Foaming and fouling of vent filter

Common Cell Retention Device (CRD) Challenges:

- Membrane fouling, insufficient throughput and process duration
- Product retention (sieving)
- Poor scalability and/or offering range
- Added complexity and cost of equipment and multiple filters

The Mobius® iFlex Bioreactor with the Cellicon® Perfusion Solution was developed to address these challenges

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Mobius® iFlex Bioreactor

- Choose between fed-batch and perfusion configurations, or **add-on later**
- **Wide range of oxygenation capabilities**, suitable for low and ultra-high cell densities, at any scale
- **High-performance film** to reduce leaks
- Available with an integrated **deltaV software** control platform, configured with application-relevant sensors and capabilities (loops, alarms, trends, recipes)

Cellicon® Filter

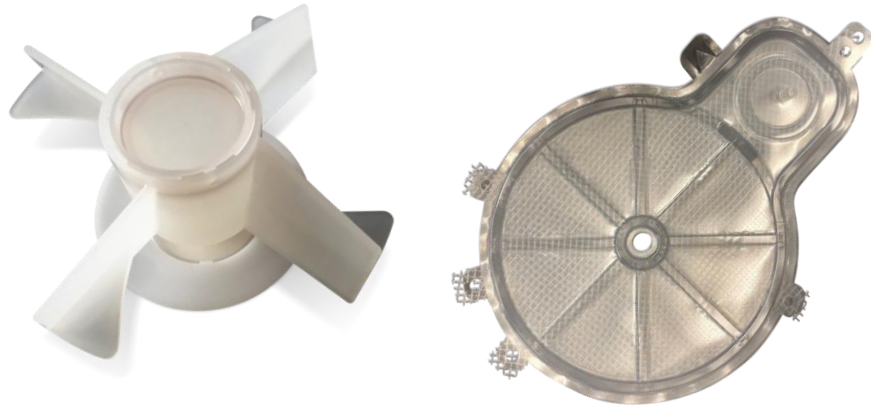
- Flat-sheet design to ensure **linear scalability** and offering to match bioreactor sizes
- **Single-use assemblies** provided with integrated components
- Microfiltration membrane provides **improved throughput and sieving**
- Available **integrated** with Mobius® iFlex Bioreactor or **standalone** with Mobius® Cell Retention System



This presentation will focus on two enabling features that enhance performance

1

Bioreactor Oxygenation



2

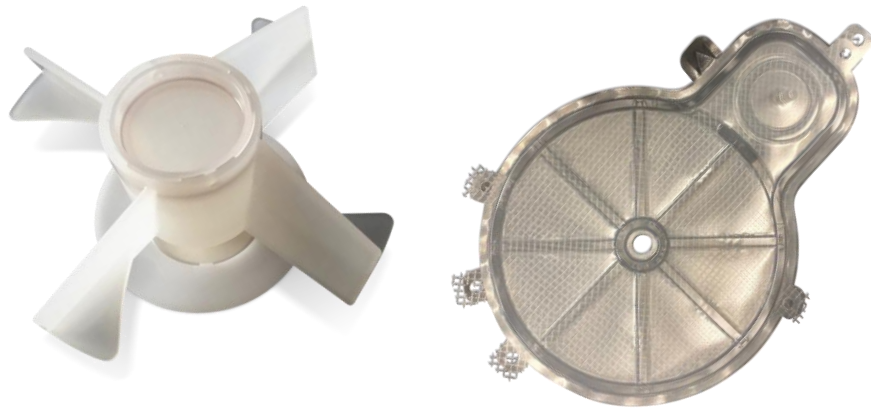
Cell Retention Performance



First, we will share details on the design and performance of a new bioreactor technology

1

Bioreactor Oxygenation



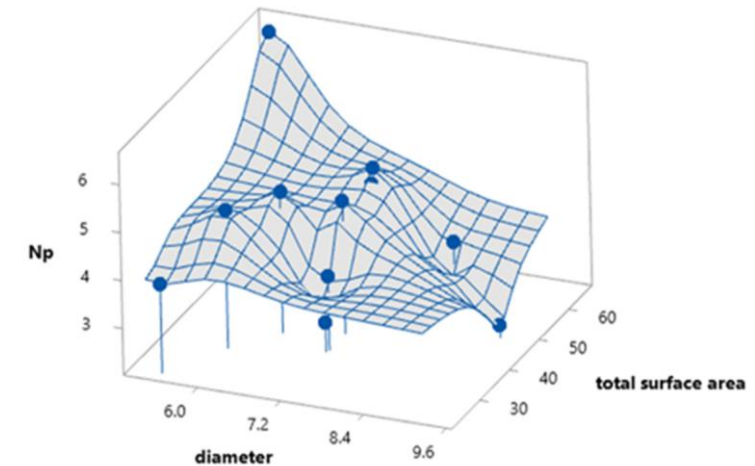
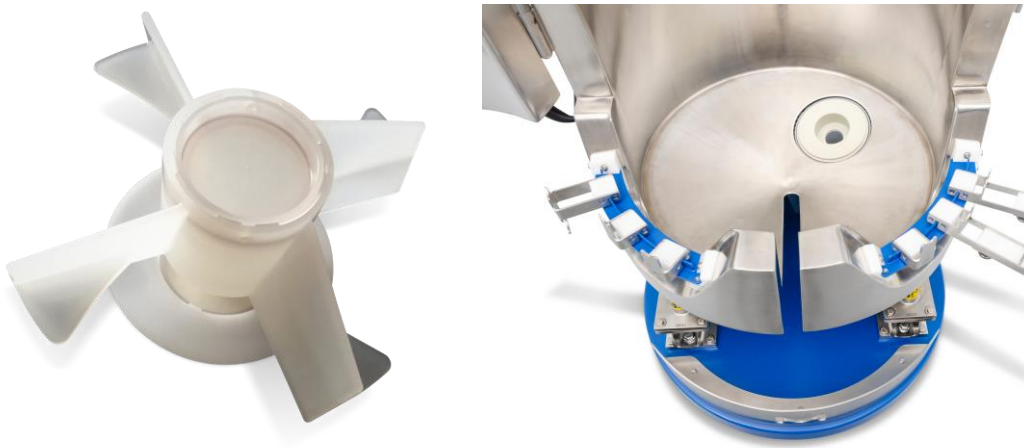
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Cell Retention Performance

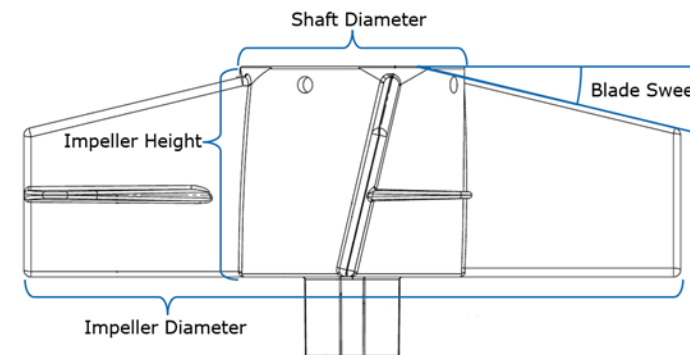


Levitronix® Motor with optimized impeller design was selected for scalable performance

- Utilizing Levitronix® motor capabilities (torque), a power number model-based approach was used to predict optimal impeller designs to achieve performance targets (tip speed, P/V, mixing time)
- Appropriately selected impeller dimensions to give a constant power number of ~ 3.6 across scales



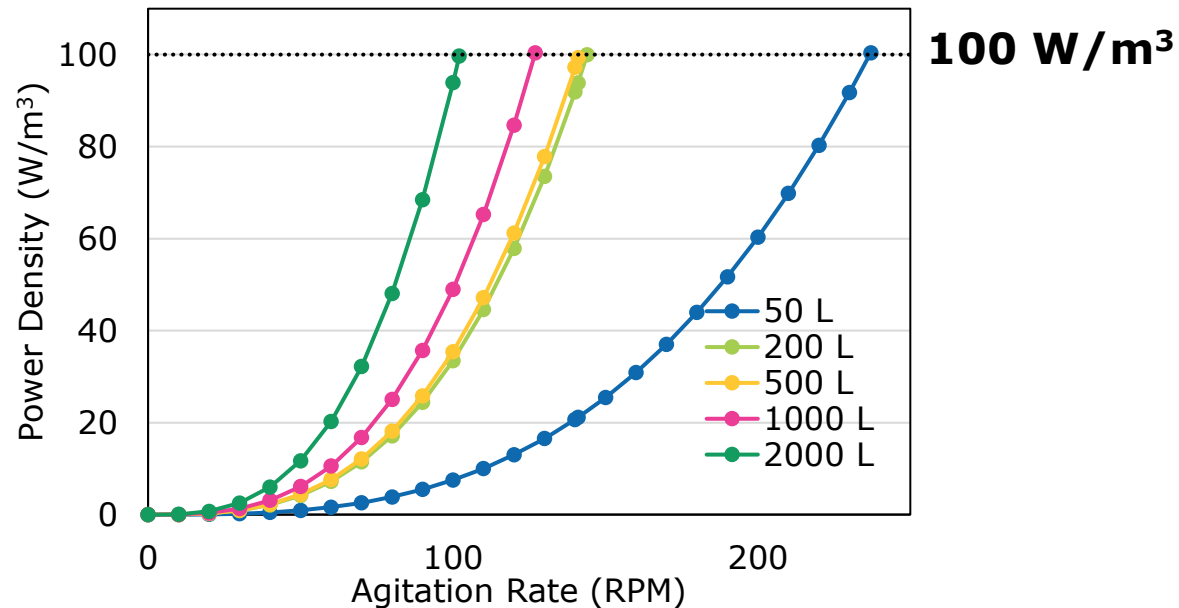
Scale (L)	Diameter (in)	Height (in)	Blade Sweep (deg)
50	4.6	1.6	18°
200	8.3	2.6	13.5°
500	10	2.8	11°
1000	12.2	3	11°
2000	16	4.1	11°



Impeller and motor deliver high power input for improved mixing and gas transfer, while preventing cell damage

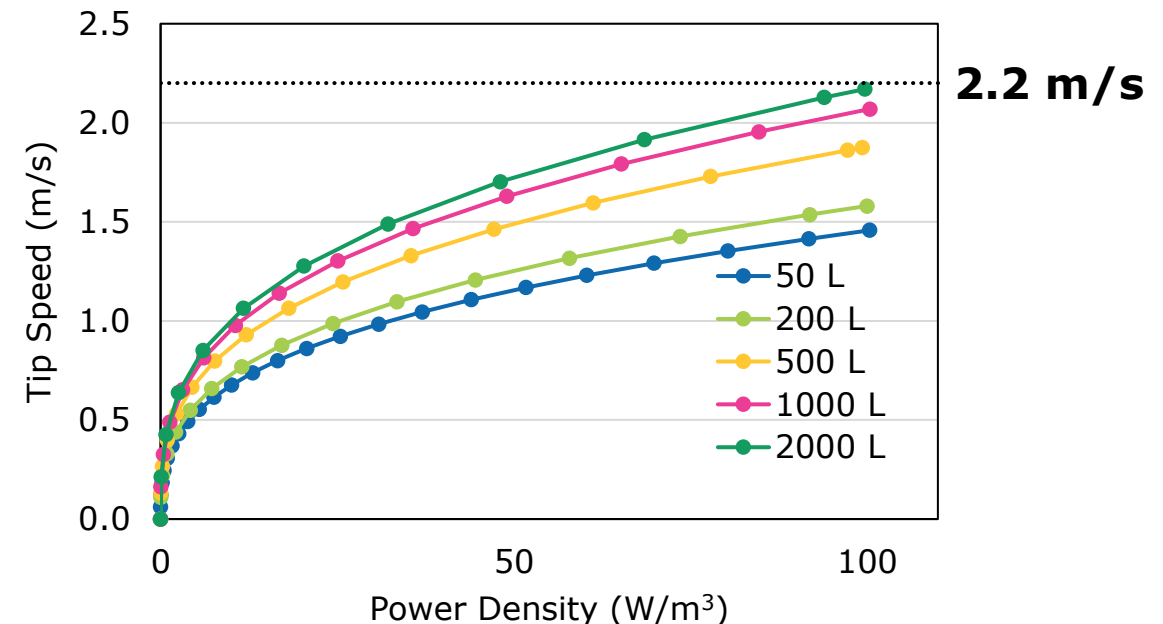
Power per Volume

- Describes energy transfer from mechanical mixing into cell culture
- Function of the power input, power number, impeller diameter, liquid volume, fluid density, and agitation rate



Tip Speed

- Indicates shear force at the outer edge of the impeller blades
- Function of the impeller diameter and agitation rate



All Mobius® iFlex Bioreactors reach 100 W/m³

All Mobius® iFlex Bioreactors remain below a tip speed of 2.2 m/s at 100 W/m³.

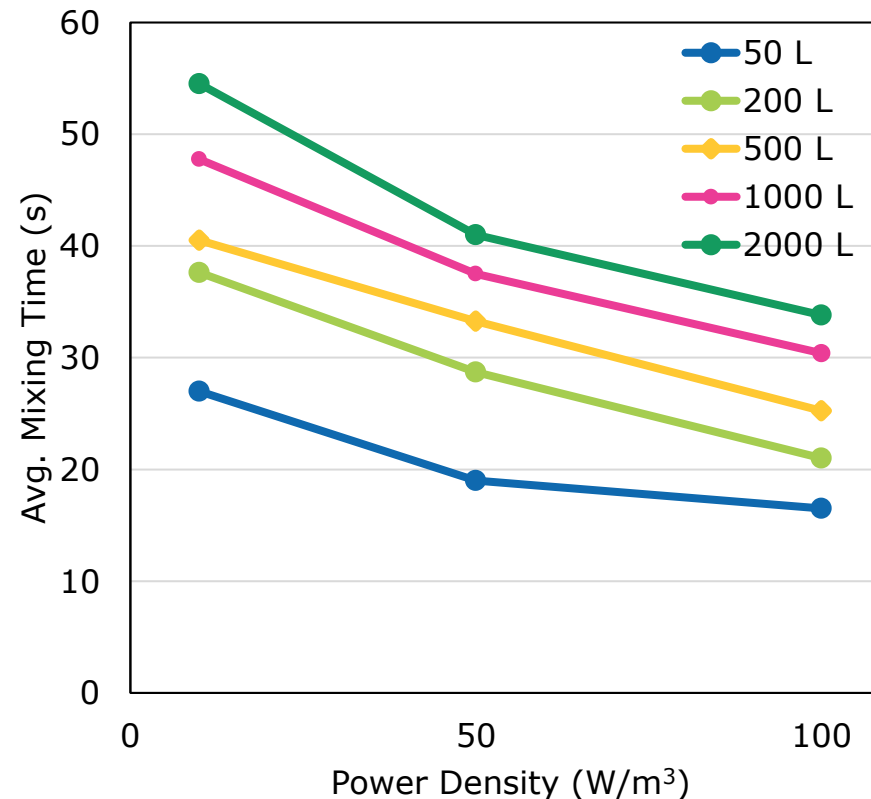
Impeller and baffle designs ensure mixing homogeneity and low mixing times

Mixing time is a critical parameter as it ensures:

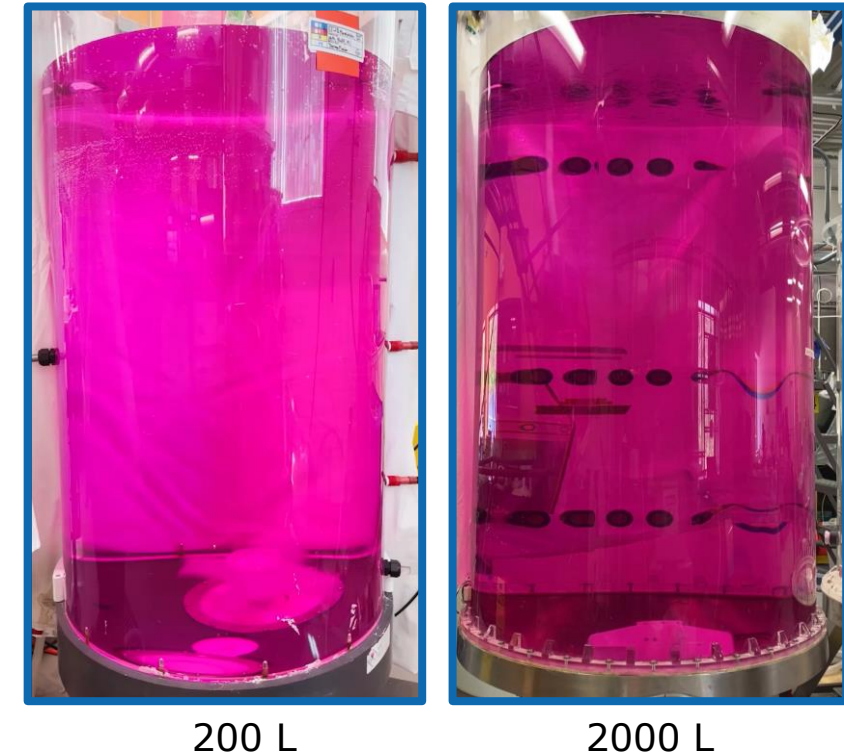
- even nutrient distribution, and
- minimal waste concentration

Mixing times were determined using a combination of pH sensor method and phenolphthalein colorimetric method.

Mixing Times



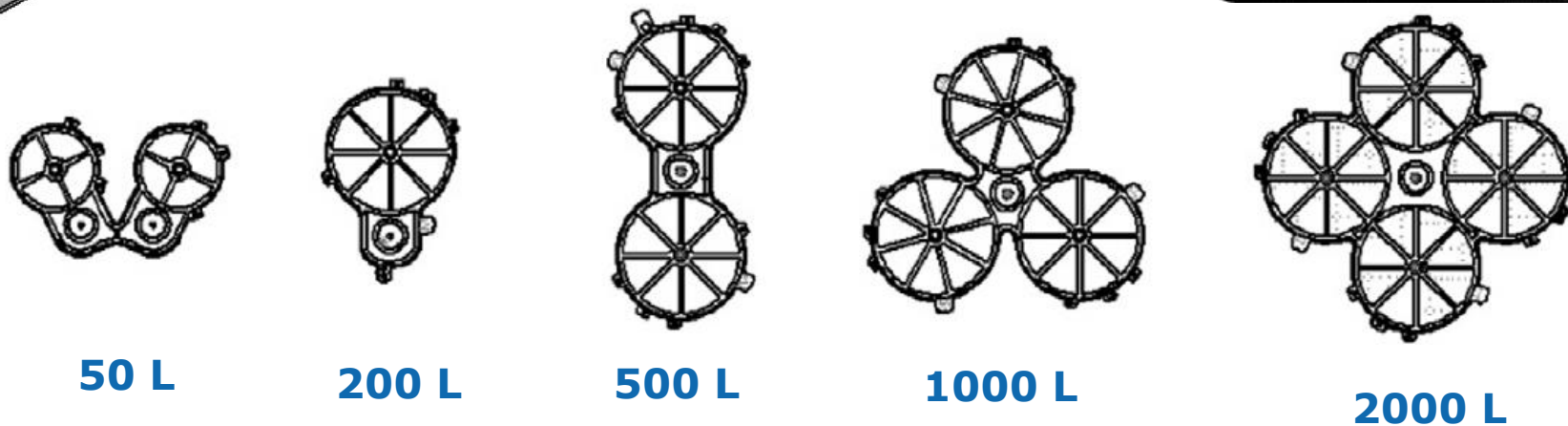
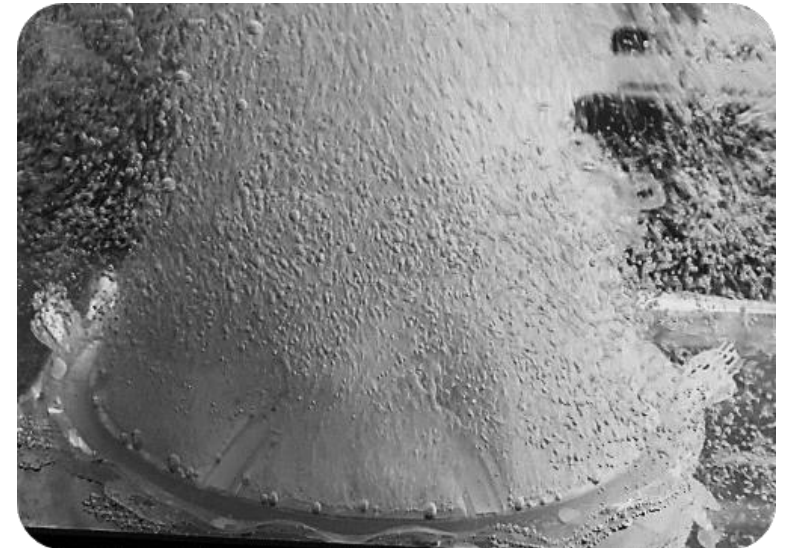
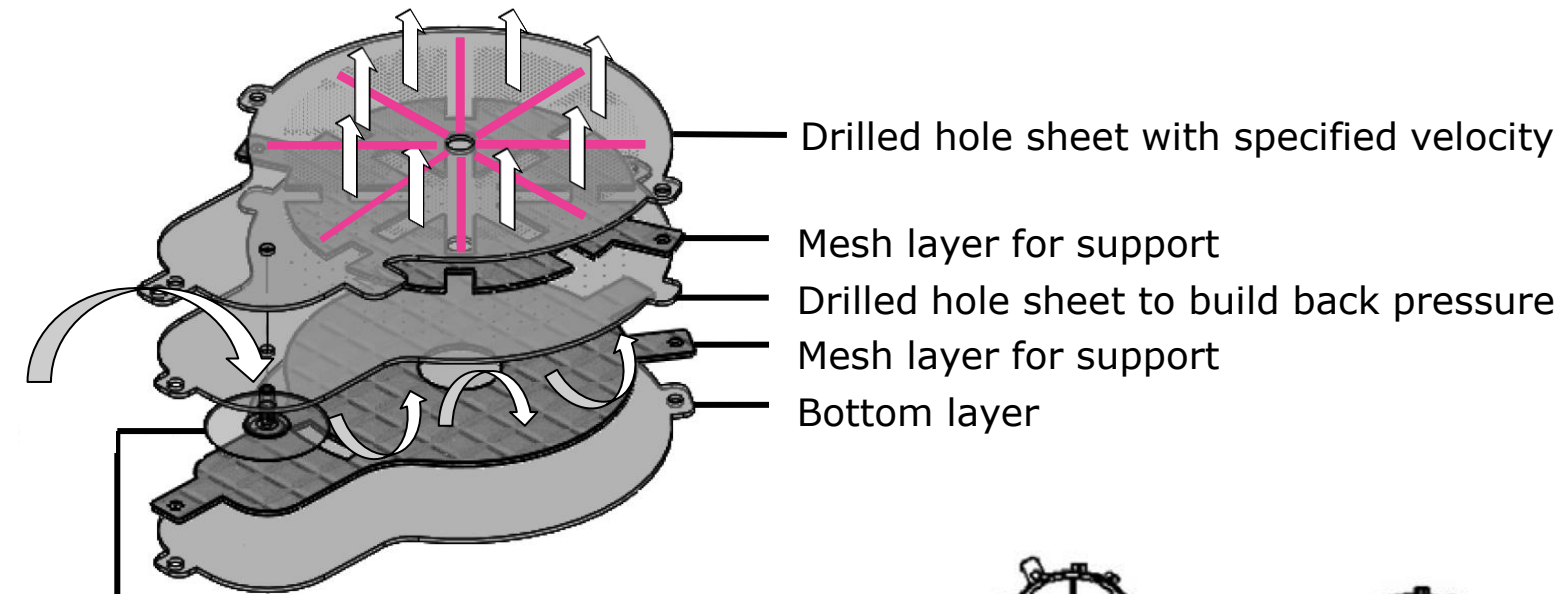
Mixing with X-Baffle (100 W/m³)



All Mobius® iFlex Bioreactors achieve mixing times <34 sec at 100 W/m³.

Drilled hole spargers provide optimal gas distribution at two different bubble sizes

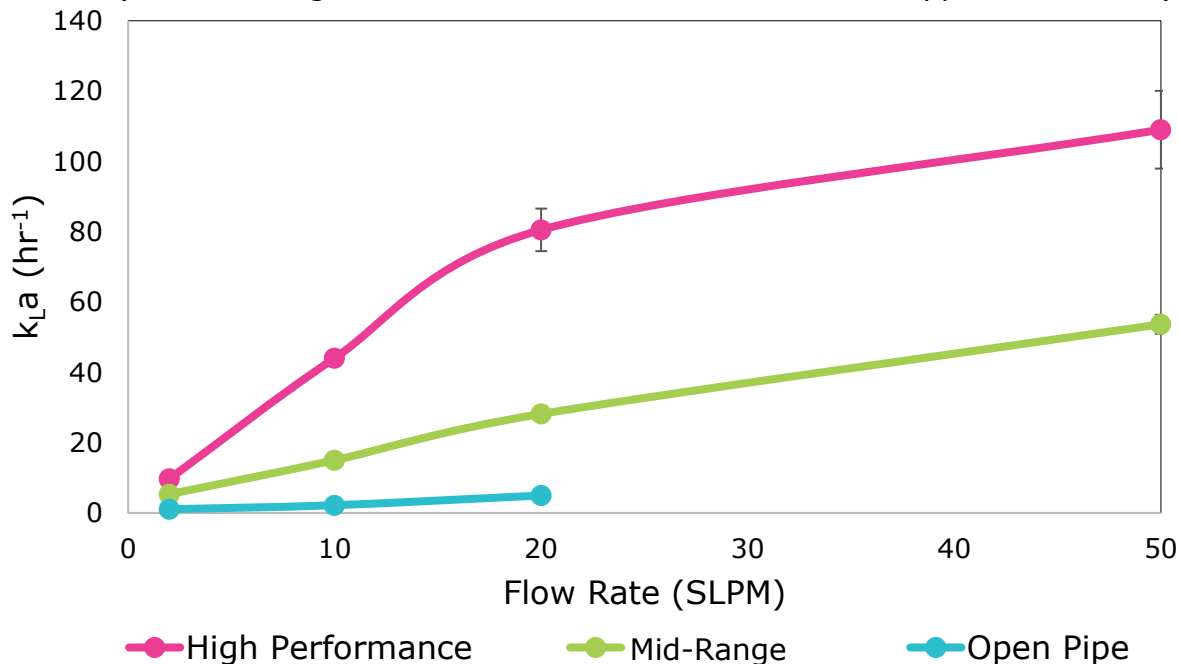
25 Micron (High Performance Sparger) and 150 Micron (Mid-Range Sparger)



Multiple sparger options allow for process-optimized gassing strategies with predictable k_La across scales

Spargers Offer Range of k_La

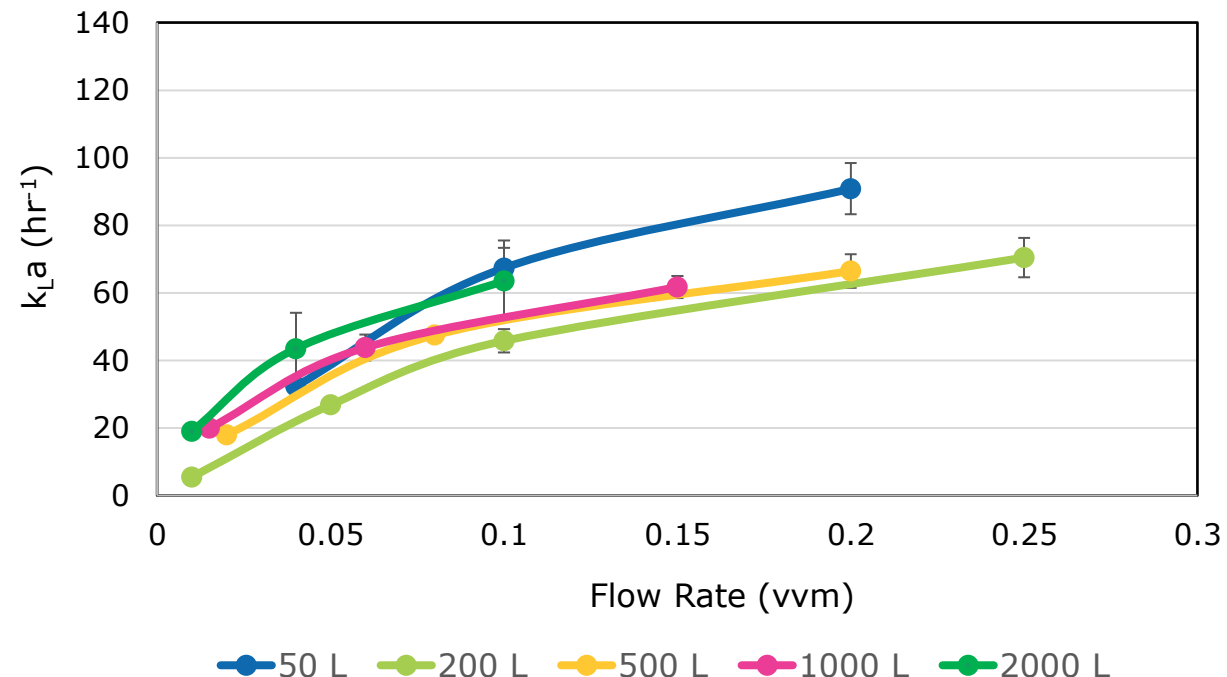
200L Bioreactor, 100 W/m³, Mock Media Buffer
(1X PBS + 4 g/L EMPROVE® 188 Poloxamer + 50-100 ppm Antifoam C)



High Performance, Mid-Range, and Open Pipe spargers achieve k_La of > 100, 50, 5 hr⁻¹ respectively

k_La Scalability with High Performance Sparger

Acrylic Tanks, 100W/m³, 1X PBS



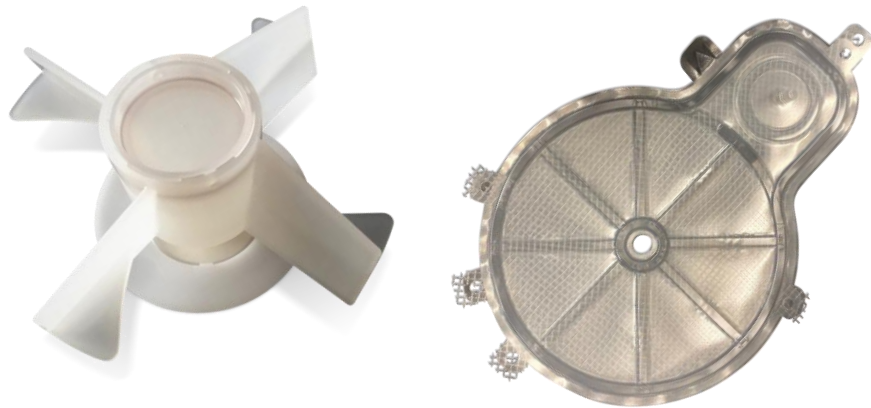
Scalability of k_La has been confirmed: All Mobius® iFlex Bioreactors achieve k_La > 60 hr⁻¹ at 100 W/m³.

Mobius® iFlex Bioreactor oxygenation strategy enables high-performance and flexibility for all process types

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1

Bioreactor
Oxygenation



2

Cell Retention
Performance



Cellicon® Filters are designed for robust performance, ease-of-use, and scalability

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- Flat sheet tangential flow filter designed for **robust performance**
 - 5 Micron Durapore® membrane: High flux and low protein binding for increased throughput and product yield
- **Closed, sterile, single-use assembly** provided with all required components
 - Low-shear Levitronix® levitating centrifugal feed pump
 - Levitronix® multi-use flow sensor for recirculation flow control
 - Pressure sensors for monitoring of filter performance
 - Perfusate pump and Levitronix® Single-Use Flow Sensor for flow control (process scale only)
 - Sterile weld (all sizes) & AseptiQuik® Connectors (process-scale only)
- **Easily scales** across different process volumes
 - Lab scale: 1-3 L
 - Process scale: 50, 200, 500, 1000, and 2000 L

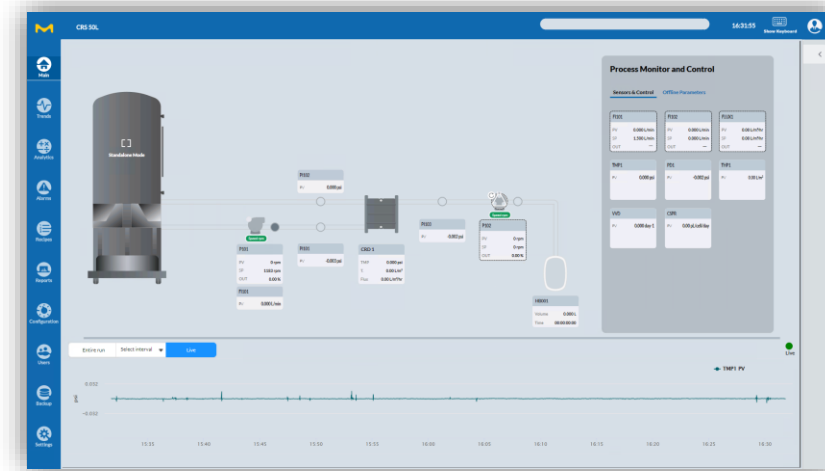


Cell retention systems provide simple, all-in-one perfusion control capabilities

Cellicon® Controller with Levitronix® Software (3L)



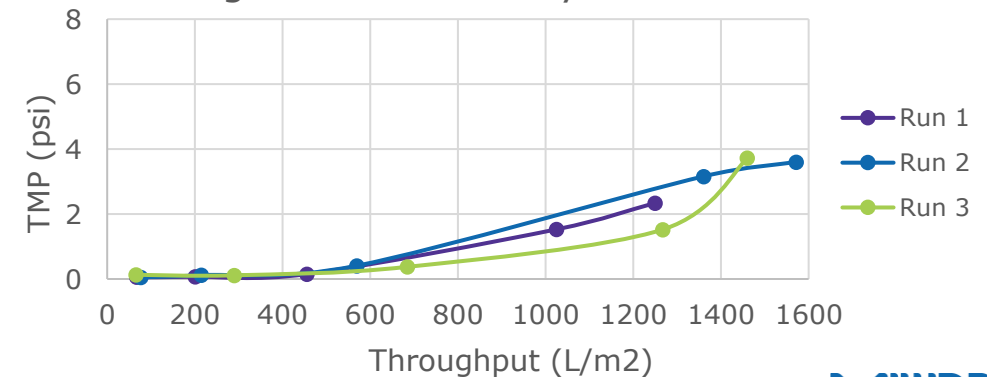
Mobius® Cell Retention System with Bio4C® ACE Software (50 – 2000L)



- Complete set up in minutes
- Robust monitoring and control without complexity
 - Allows for real-time process data, flow control & pressure monitoring
 - Calculates derived process parameters for continuous monitoring (VVDs, pressure drop, flux, throughput)
- Achieve consistency from run-to-run with reliable flow control



Fouling Rate Consistency in N-1 Perfusion



Optimization of scaling criteria ensures predictable performance from lab to manufacturing

Key Scaling Parameters:

- **Channel height and length** are constant across all sizes; **channel width** is constant across all production scales
- **Membrane area** scaled by ratio of membrane area to bioreactor volume
- Max **perfusion/permeate rate** (2 VVDs) scaled by flux across membrane (up to 22 LMH)
- **Crossflow rate** scaled by shear rate on membrane surface (2500 s^{-1})
- Levitronix® PuraLev® **Recirculation Pump** size selected to maintain RPM range
- **Tube set dimensions** are sequentially increased



3L 50L 200L 500L 1000L 2000L

	3L	50L	200L	500L	1000L	2000L
	PD/Lab	Production				
Membrane Area (m ²)	0.01	0.2	0.8	1.9	3.8	7.6
Recommended Crossflow (L/min)	0.08	1.57	5.7	14	28	2 x 28
Levitronix® Pump	i30 SU	i100 SU	600 SU	600 SU	2000 SU	2 x 2000 SU

Recirculation pumps selected to deliver required flow rates while minimizing cell shear

A 50L bioreactor was operated in batch/re-batch mode with a recirculation loop to mimic the setup for a 1000L Cellicon® Filter Assembly. The 600SU and 2000SU pumps were tested at relevant flow rates for the 1000L filter (28 LPM target). Indicators for cell shear (growth rate/doubling time, LDH, cell size, productivity) were monitored through the process and compared to data for known good and bad conditions of controlled pump speed at bench scale.

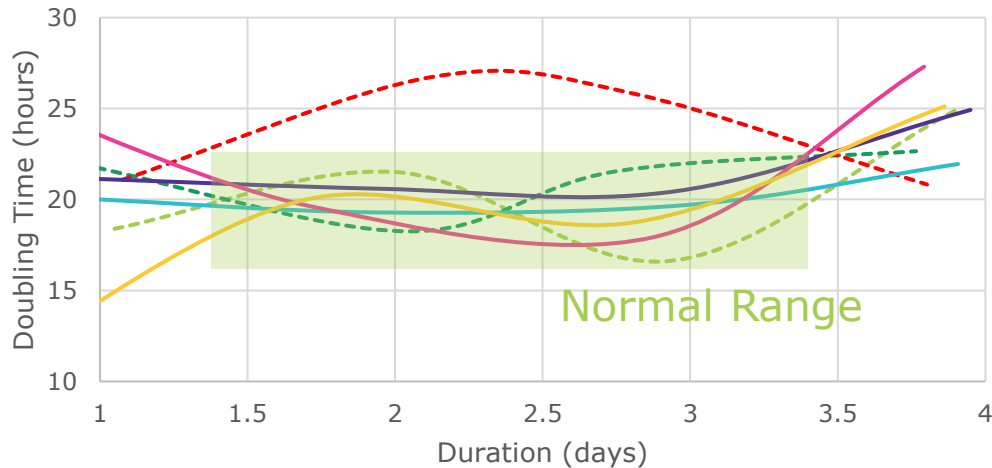
Known "good" conditions

Known "bad" condition

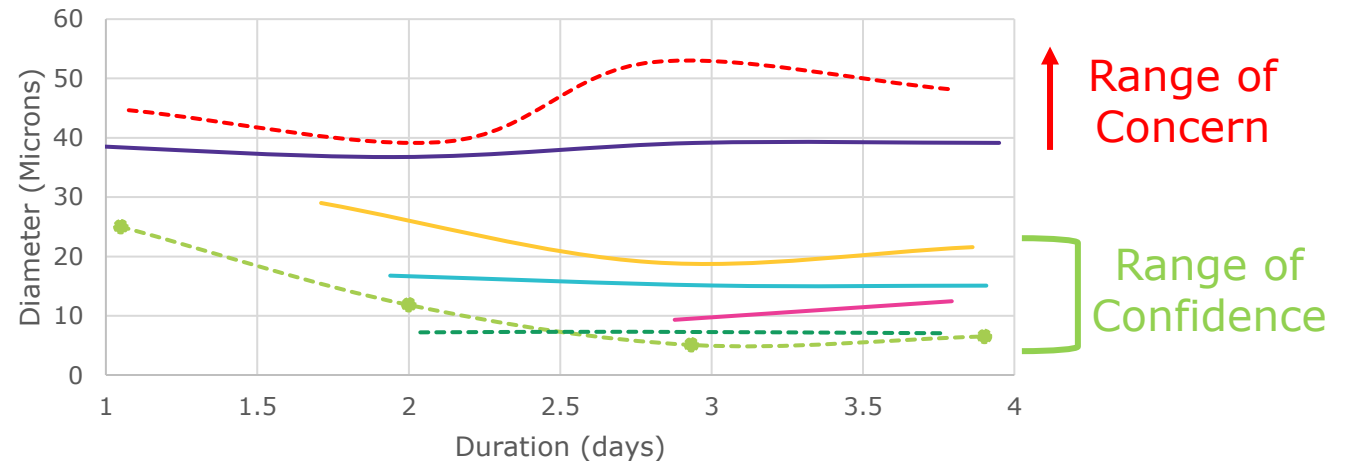
Experimental conditions, 2 psi backpressure

Pump	Volume	Pump Speed	Flow Rate
i30SU	1.7 L	3000 RPM	2.2 LPM
i30SU	1.7 L	5000 RPM	7.2 LPM
i30SU	1.7 L	7000 RPM	3.7 LPM
600SU	50 L	2856 RPM	24 LPM
600SU	50 L	3508 RPM	30 LPM
2000SU	50 L	2036 RPM	24 LPM
2000SU	50 L	2462 RPM	30 LPM

Cell Growth



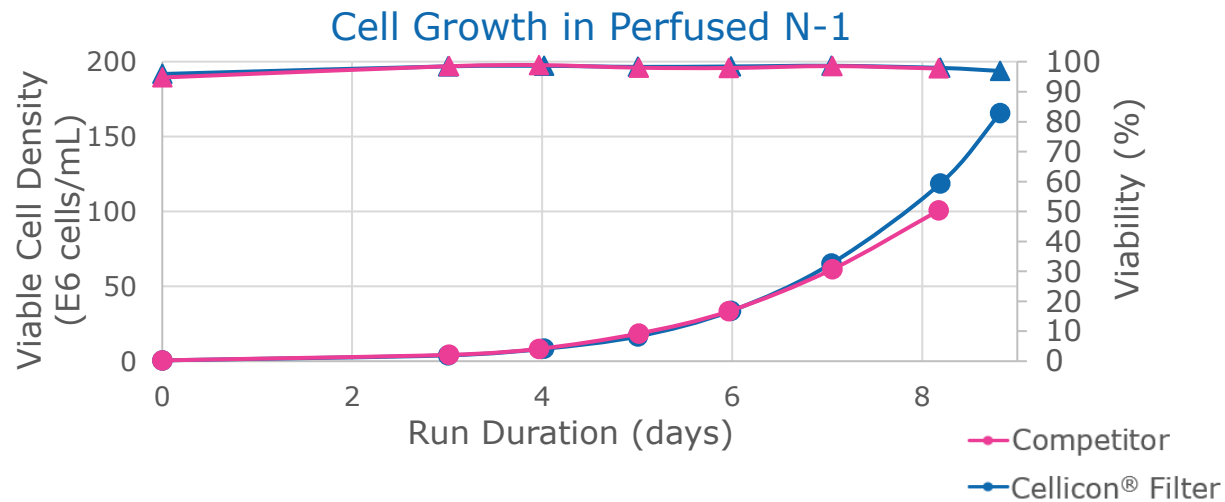
LDH per cell



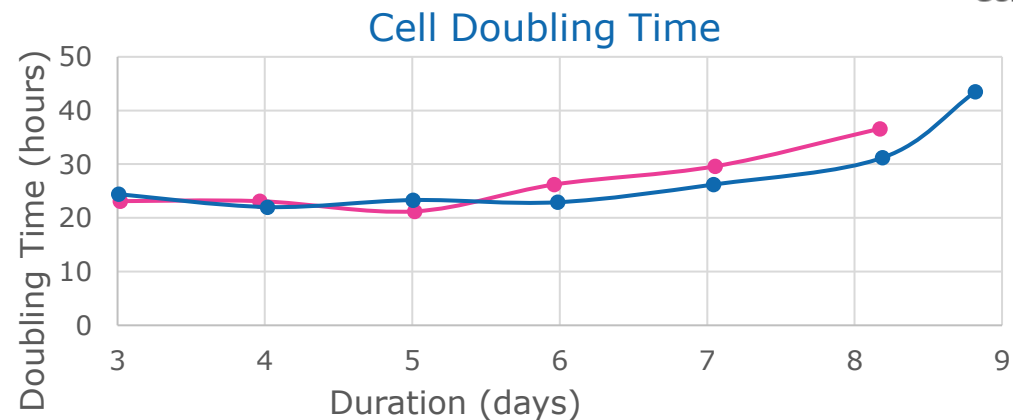
2000SU pump selected to achieve target crossflow for 1000L Cellicon® Filter while minimizing cell shear

Cellicon® Filter outlasts competitor in high cell density N-1 process while preserving cell health

Parallel 3L glass bioreactors (2.5L working volume) with the same process parameters were used to compare performance of cell retention devices, using a cell line from the CHOZN®+UCOE® Combined Platform producing an IgG1, and EX-CELL® Advanced HD Perfusion Medium.



	Cellicon®	Competitor
Format	Flat Sheet	Hollow Fiber
Area	100 cm ²	220 cm ²
Membrane	5 Micron PVDF	0.2 Micron PES
Crossflow Rate	100 mlpm	180 mlpm
Pump	Centrifugal	Diaphragm
Max Flux	25 LMH	8 LMH

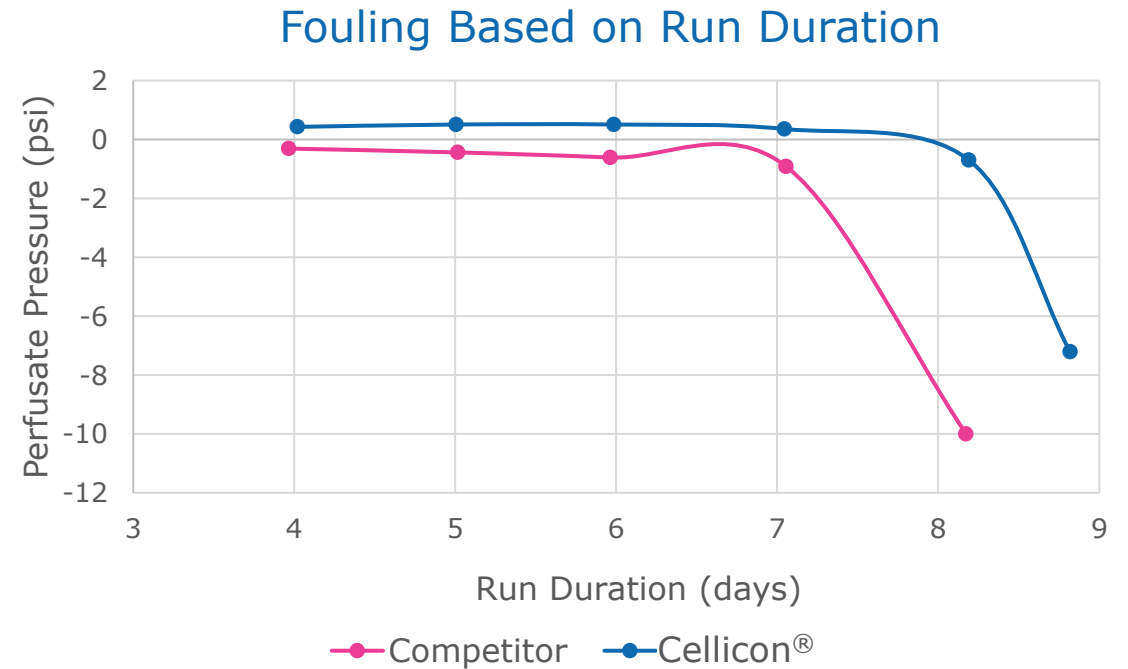
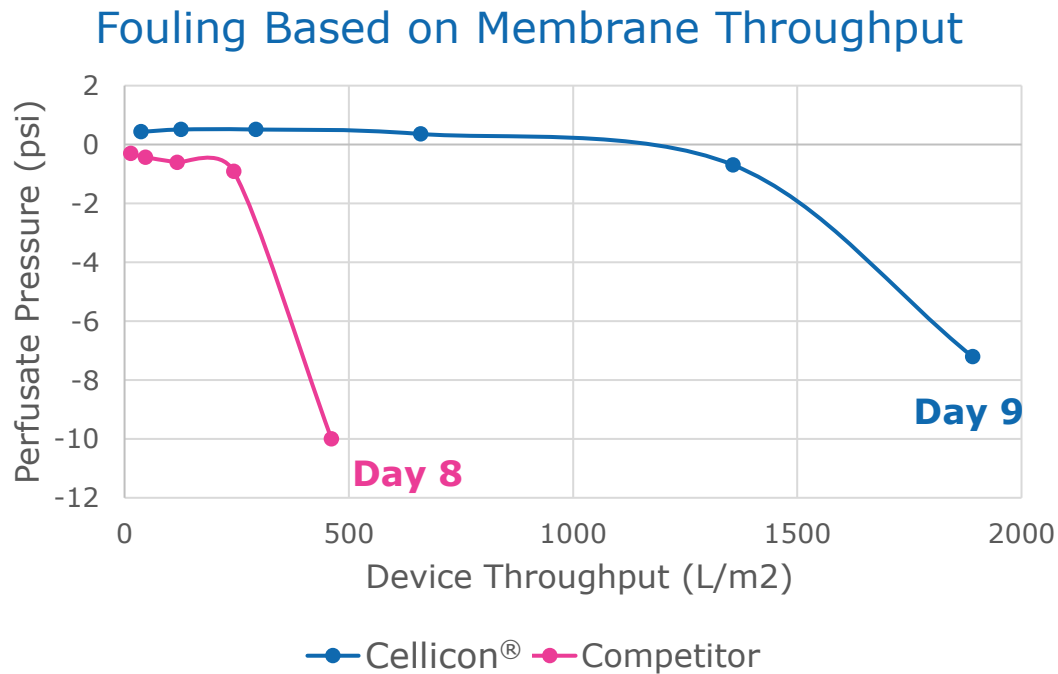


The Cellicon® Filter lasted one day longer than the competitor filter, reaching a VCD of 165E6 cells/mL

Cell growth and viability trends suggest healthy cells, confirming low shear impact of the system

Cellicon® Filter demonstrates capacity improvement from competitor filter, showing membrane efficiency

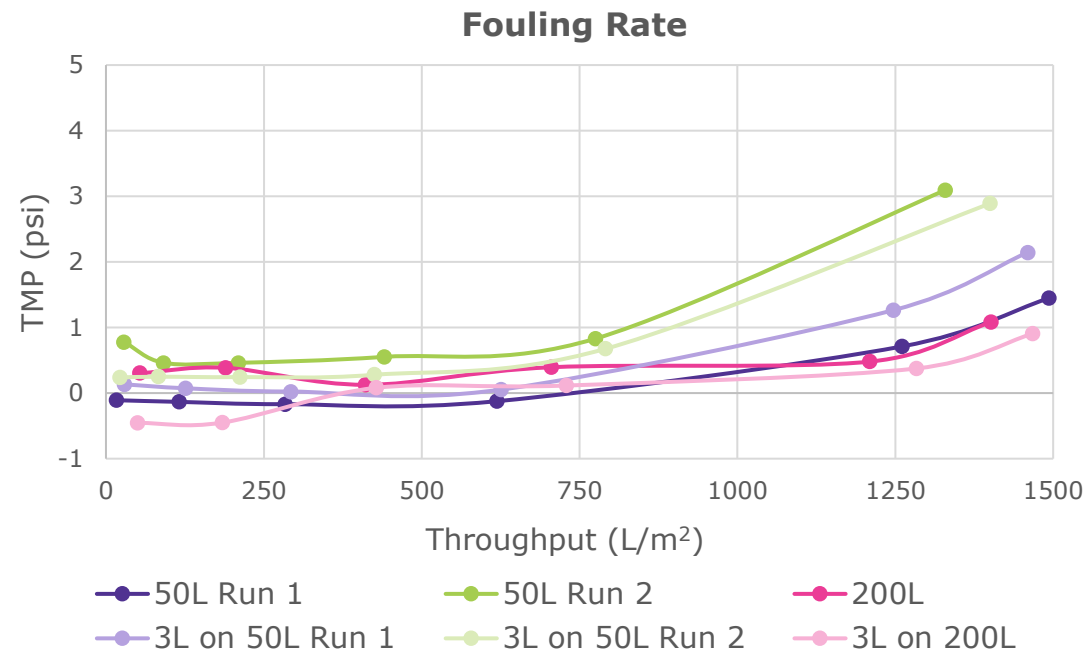
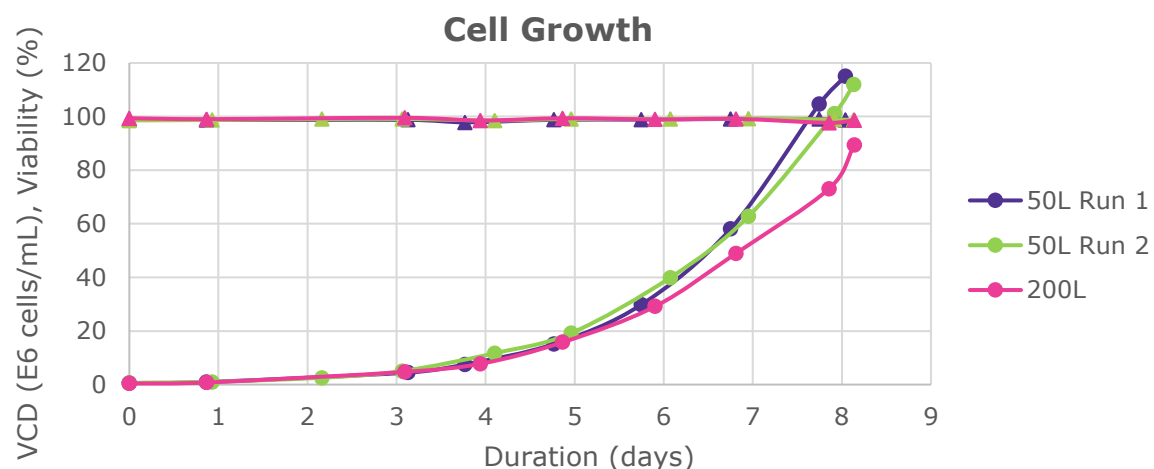
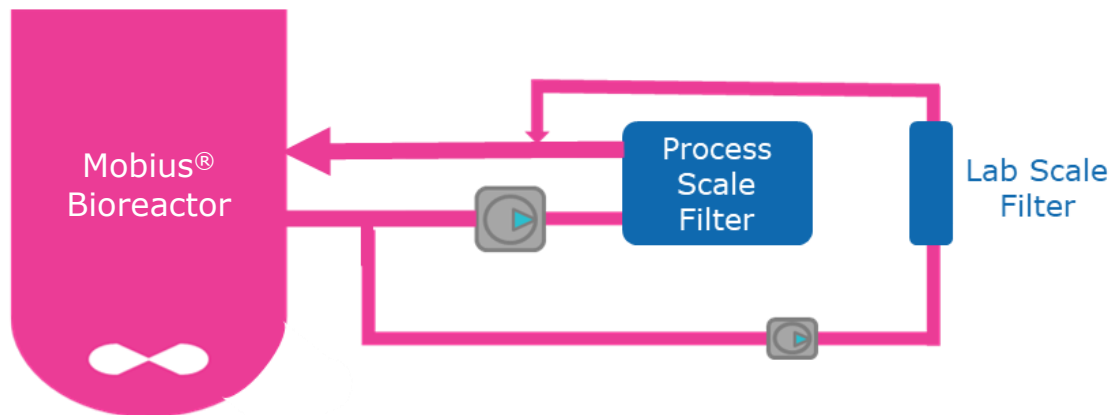
Data from the Cellicon® Filter vs. Competitor experiment in N-1 perfusion, described previously



The Cellicon® Filter delivers high throughput and improved process duration by optimizing membrane efficiency

Scalability of filter design has been confirmed in cell culture

An N-1 perfusion run using CHO-S cell line was evaluated in bioreactors at 50 & 200L working volumes. In each run, a process scale filter was evaluated in parallel to a lab-scale filter, operated at matching flux.



Consistent fouling trends observed from lab- to process-scale

Mobius® iFlex Bioreactor and Cellicon® Filter optimize performance for any upstream process

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**Bioreactor
Oxygenation**



**Cell Retention
Performance**

Thank you!

Acknowledgments

Samantha Whitney	Alison Dupont
Haley Matthews	Adam Covino
Terence MacCorvin	Joe Muldoon
Paola Amable	Nimesh Vaghela
Joseph Sinagra	Derek Silva
Marisa Maher	Lilia Vinskus
Amy Wood	Misha Kozlov
Jonathan Cain	Amy Mei
Gujie Mi	Sualyneth Galarza
Diana Perez	

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