Producing value

The Application of Levitronix Pumps in the Scale up of an Intensified Perfusion Process

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- Founded 1885 in Ingelheim, Germany
- Focus on Human Pharmaceuticals, Animal Health and Biopharmaceutical Contract Manufacturing
- Around 50,000 employees worldwide
- 181 affiliated companies worldwide
- 16 production facilities (human pharmaceuticals) in 11 countries
- Four R&D sites worldwide
- Net sales of around EUR 18.1 billion
- R&D expenditure of EUR 3.1 billion

Our Development and Production Network Spans the Globe

Biberach, Germany,
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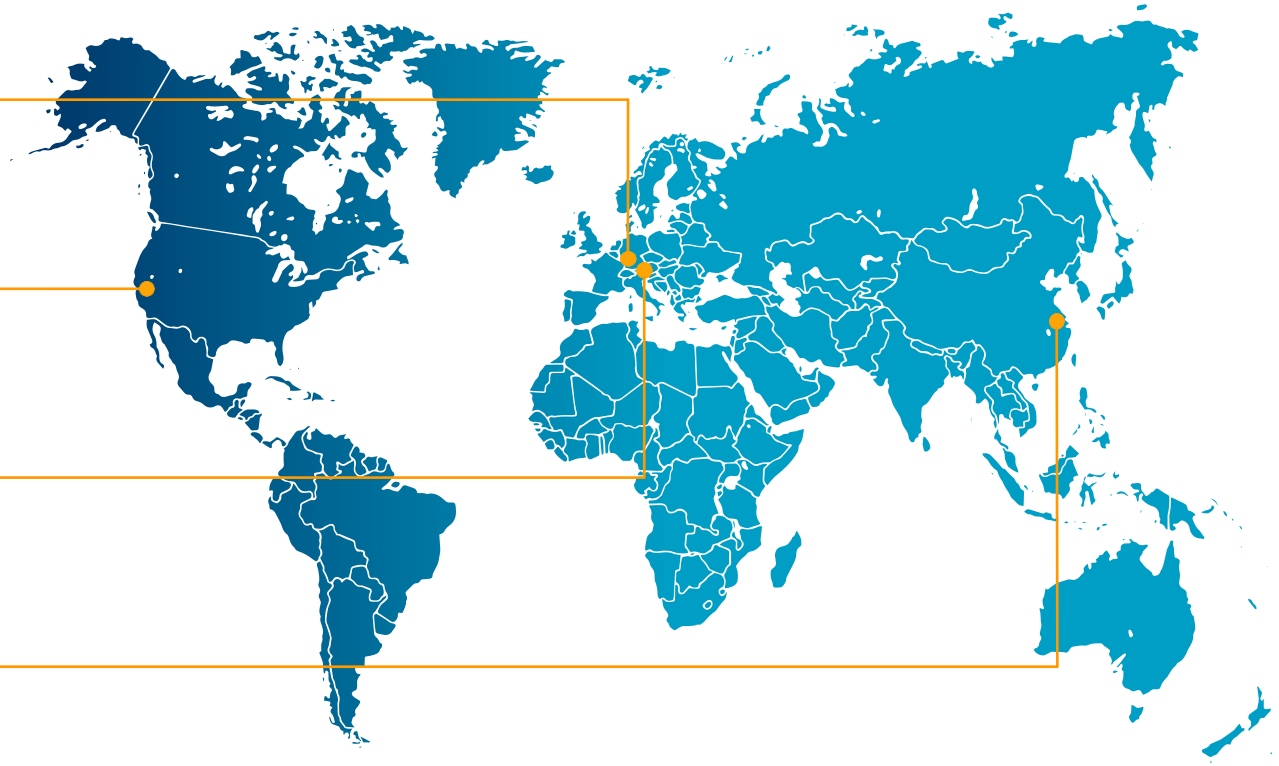
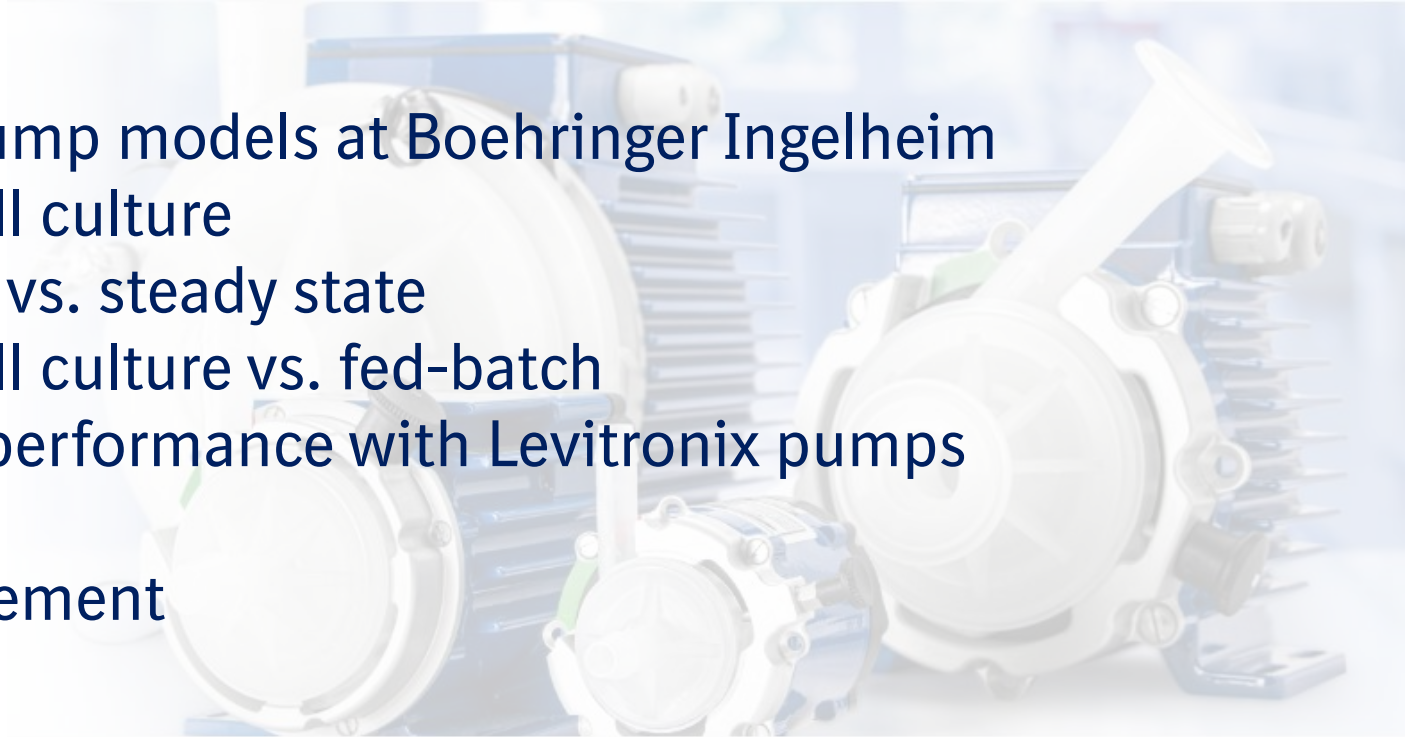


Agenda



- Levitronix pump models at Boehringer Ingelheim
- Perfusion cell culture
  - Dynamic vs. steady state
- Perfusion cell culture vs. fed-batch
- Cell culture performance with Levitronix pumps
- Conclusion
- Acknowledgement

Transition History to Levitronix Pumps

Shear contributions to cell culture performance and product recovery in ATF and TFF perfusion systems

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ABSTRACT

Achievement of a robust and scalable cell retention device remains a challenge in perfusion systems. Of the two filtration systems commonly used, tangential flow filtration (TFF) systems often have an inferior product sieving profile compared to alternating tangential flow filtration (ATF) systems, which is typically attributed to the ATF's unique alternating flow. Here, we demonstrate that observed performance differences between the two systems are a function of cell lysis and not the alternating flow as previously thought. The peristaltic pump used in typical TFF perfusion systems is shown to be the single major contributor to shear stress and cell lysis. Replacing the peristaltic pump with a low shear centrifugal pump brought cell growth, cell lysis, particle concentration, and product sieving in a TFF perfusion system to levels comparable with that of an ATF. These results provide a correlation where poor product sieving can be partially explained by high shear in cell retention systems and demonstrate that low shear TFF systems are a feasible alternative to ATF systems.

- ATF : Alternating Tangential flow filtration
- TFF : Tangential flow filtration

- Traditionally ATF was favored over TFF systems due to better performance
- Replacing peristaltic pumps with low shear Levitronix pumps brought cell growth, cell lysis, and product sieving in a TFF system to levels comparable with that of an ATF

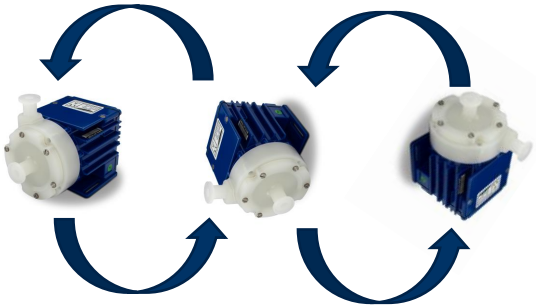
Use of Levitronix® Pumps as Recirculation Device in Perfusion

PuraLev® i30SU

PuraLev® i100MU

PuraLev® 600SU

PuraLev® 2000SU

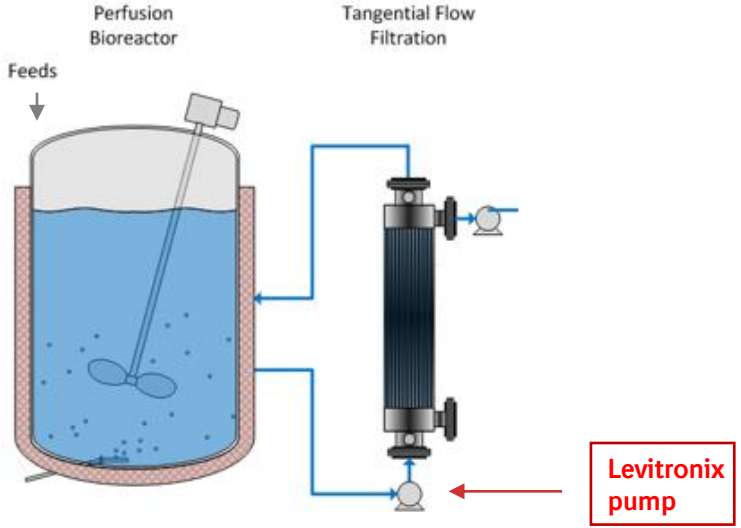


In addition to all the benefits of Levitronix pumps, the i100MU has a small footprint, allowing its use in small spaces.

Use of Levitronix Pumps Across BI Network

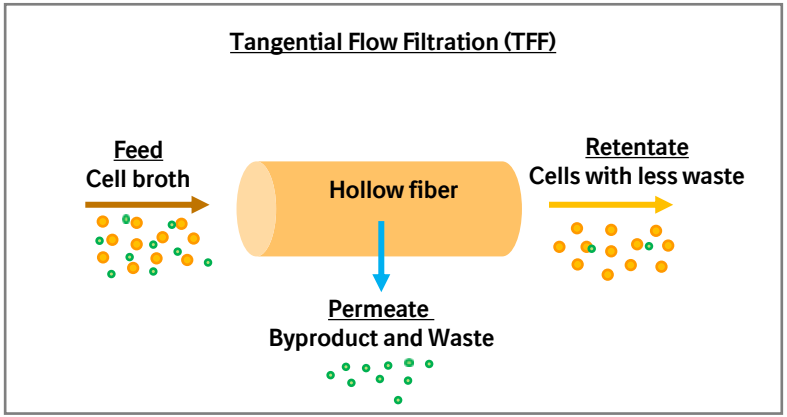
Levitronix Pump	Scale	BI Site
PuraLev® i30SU	Bench scale	✅ BIFI ✅ BIB
PuraLev® i100MU	2L ,5L, 50L & 100L	✅ BIFI ✅ BIB ✅ BI Shanghai
PuraLev® 600SU	100L & 400L	✅ BIFI ✅ BIB
PuraLev® 2000SU	500L	✅ BIFI ✅ BIB

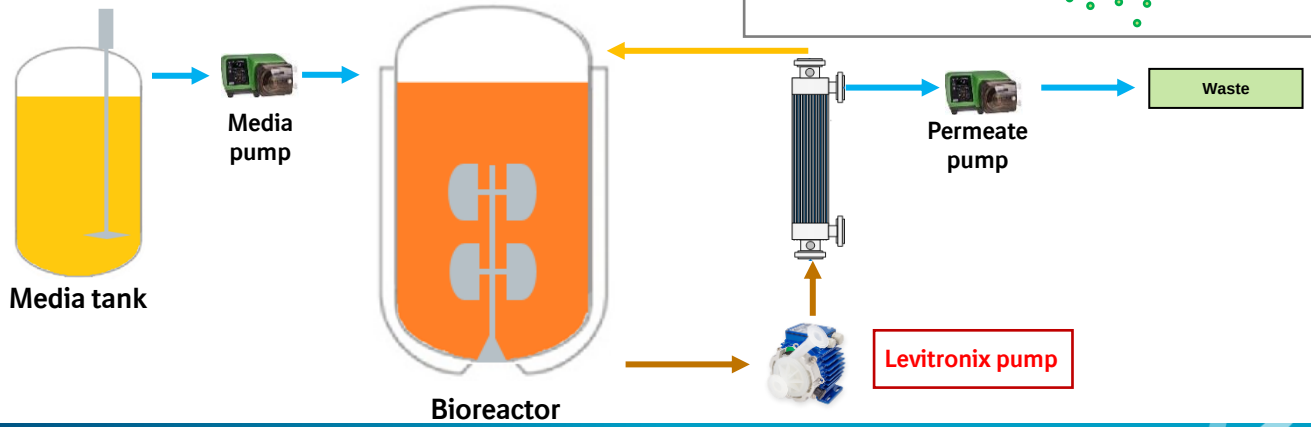
BIB : BI Biberach
BIFI : BI Fremont



What is Perfusion Cell Culture?

- During cell cultivation, byproduct and waste are continuously removed through Tangential Flow Filtration (TFF)
- Concurrently, fresh nutritional media is added continuously
- Perfusion improves cell growth, viability, and extends culture duration

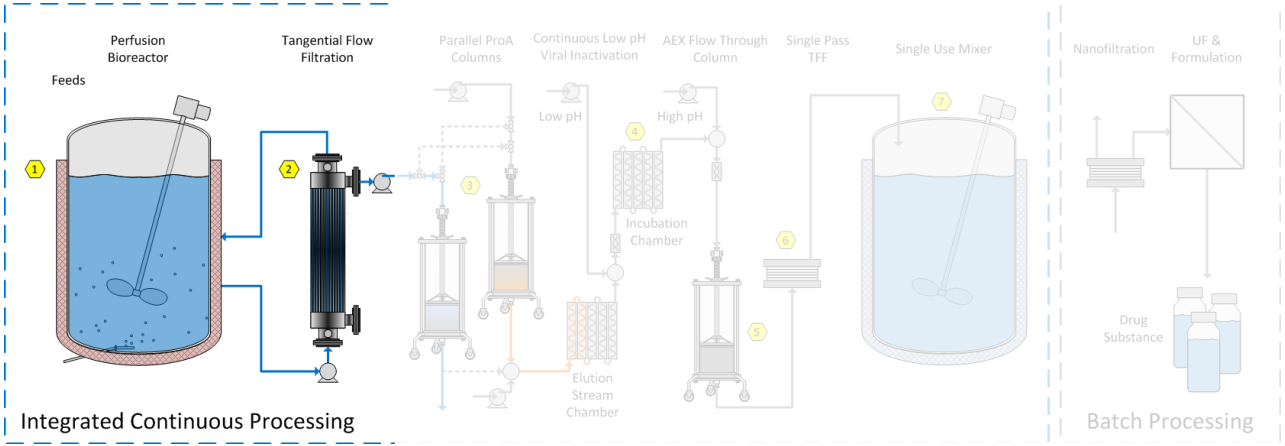




Advantages of Perfusion over Fed-batch Cell Culture

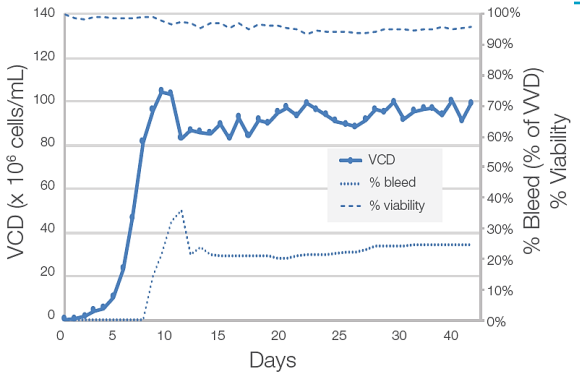
- **Common advantages of perfusion over fed-batch mode**
  1. Enhancement in productivity with highly intensified culture
  2. Retain the desired product quality with continuous harvesting for unstable molecules
  3. Significantly reduces facility footprint and enhances modularity (e.g., single-use technology)

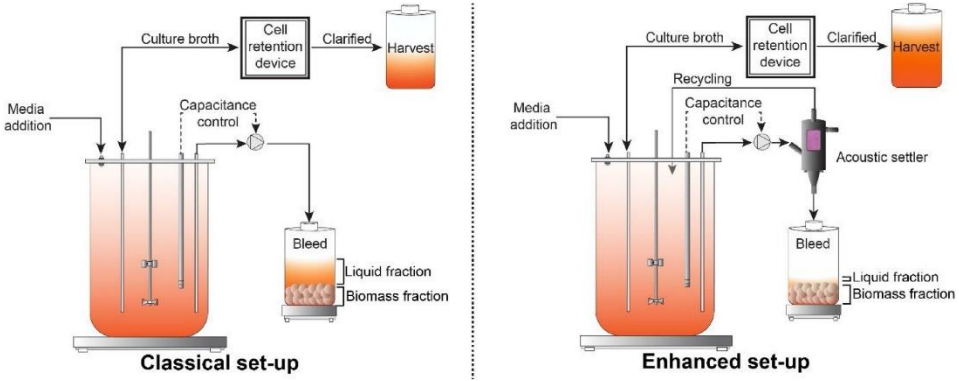
Upstream perfusion + downstream purification = “maximum benefits”



Cell Bleed : Traditional Strategy to Maintain Steady-State Culturing

- Cell bleed: removal of cells to maintain **healthy** and **steady** culture environment
- Generally, cause a significant waste of product and should be **minimized** for an efficient process
- Additional technologies are being implemented to **recover** harvest fluid (with limitations)

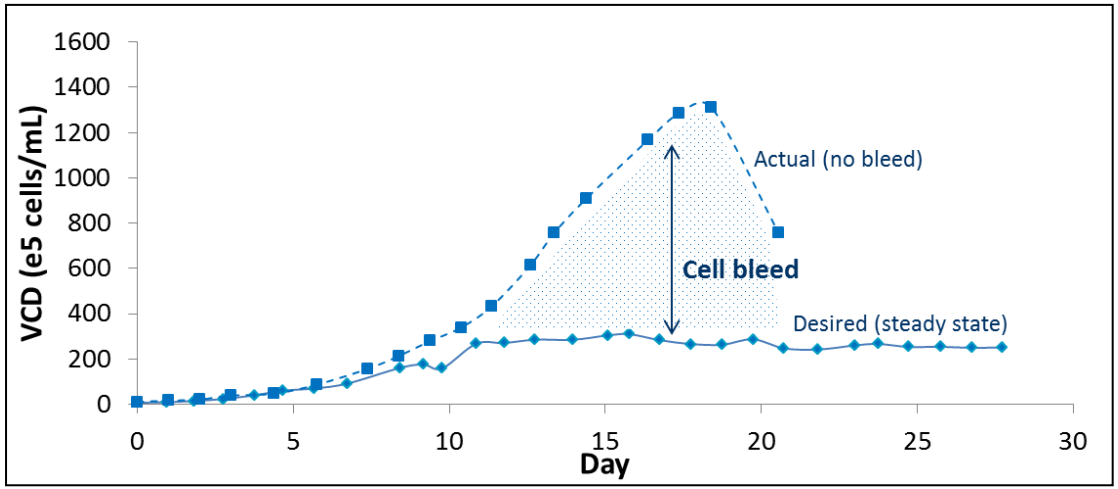




Bielser et al., *Biochem. Eng. J.* **2021**

Dynamic Perfusion vs. Steady-State Perfusion

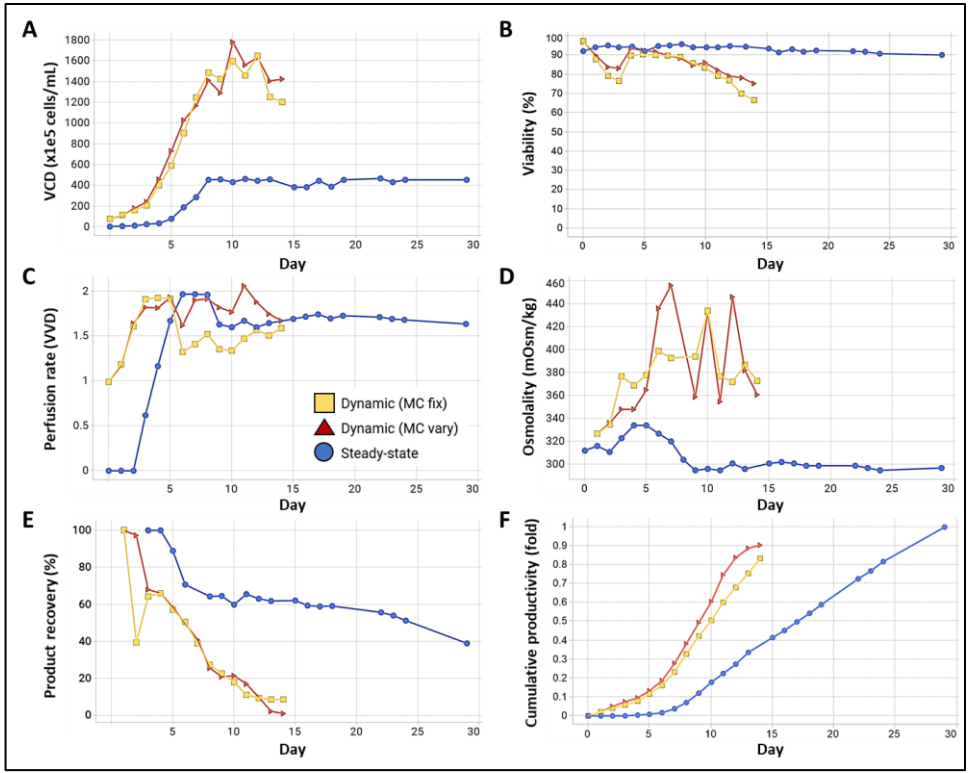
- Dynamic perfusion: achieve high peak viable cell density (VCD) without cell bleeding
- Rapid decline in cell density may lead to a pre-mature run termination
- Difficult to achieve with conventional 1x perfusion media (require decoupling osmolality and perfusion rate)



Can we maintain the culture in a "prolonged intensified state" without cell bleed?

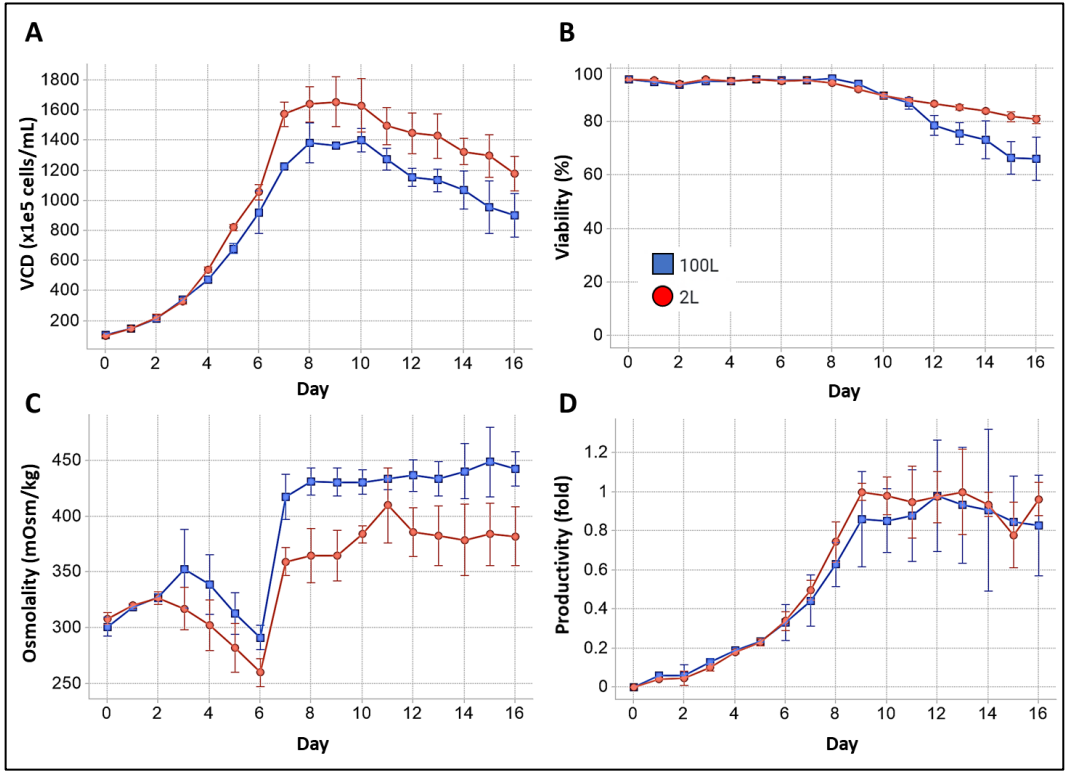
Dynamic Perfusion vs. Steady-State Perfusion

- Support ultra-high cell density in dynamic perfusion with titer enhancement
- Osmolality-based growth control – eliminates cell bleed and product loss
- Fluctuation in residual osmolality due to deviation from media concentrate (MC) feeding device
- Daily adjustment by operator could be labor intensive and non-GMP friendly



Promising Performance across Scales

- Comparable process performance across scales
- Stabilize residual osmolality with less deviation in product quality
- GMP-friendly and suit for future scale up to manufacturing scale

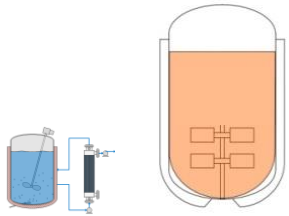


Perfusion and Fed-batch Comparison

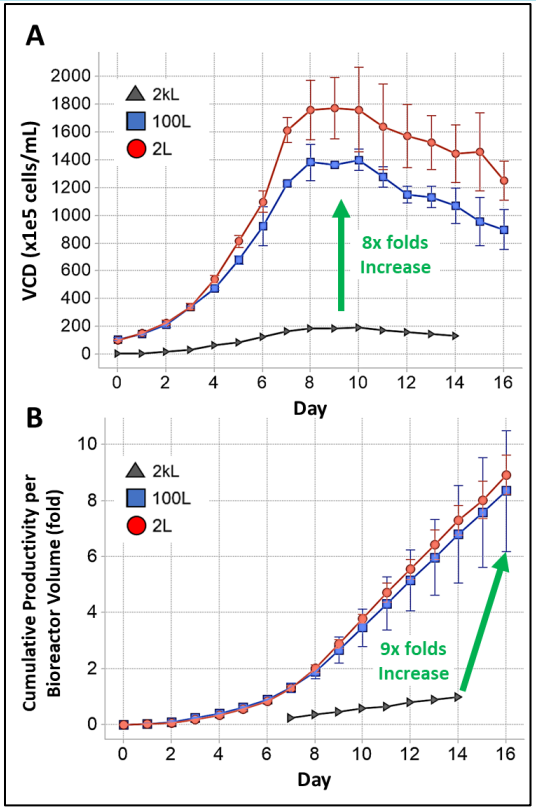
Superiority in both growth and productivity

- ✓ Achieved peak VCD of 150 × 10^6 cells/mL
- ✓ 9x folds enhancement in productivity
- ✓ Comparable product quality

A 2kL *stainless steel bioreactor* run can be replaced by 2x *100L single-use bioreactor* runs by yield!



Conditions	100L SUB (n=2)	2kL SS (n=6)
Cumulative productivity	9.2x	1x
Total harvested product	1x	2.2x



Conclusion

- Levitronix pumps have enabled us to run perfusion process across all scales with no cell culture impact

- Implementation of Levitronix pumps in 3 major Boehringer Ingelheim facilities: Fremont, Biberach and Shanghai

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Thank you!

Q & A