Fall 11-2-2015

Upstream perfusion process: Back to the future

Jean-Marc Bielser
EMD Serono, jean-marc.bielser@merckgroup.com

Henri Kornmann
EMD Serono

Herve Broly
EMD Serono

UPSTREAM PERFUSION PROCESS

Back to the future

Jean-Marc Bielser
ICB II, Berkeley, 2nd November 2015

EMD SERONO

EMD Serono is a
business of Merck KGaA,
Darmstadt, Germany

WE ARE MERCK – THE ORIGINAL

In 1887, Merck opened its own office in New York, which gave rise to the subsidiary Merck & Co. three years later. As a result of World War I, this subsidiary was expropriated in 1917 and has been an independent company ever since 1917.

Merck – the original holds the global rights to the Merck name and brand.

Exceptions are Canada and the United States, where we do business as EMD Serono, EMD Millipore and EMD Performance Materials.

3 business areas
What we do

Prescription medicines to treat, for example, cancer, multiple sclerosis and infertility, **over-the-counter pharmaceuticals** for everyday health protection or to provide fast relief of colds and pain, as well as innovations in the areas of **allergies** and **biosimilars**.

Innovative **tools** and **laboratory supplies** for the life science industry that make **research** and **biotech** production easier, faster and more successful.

A wide range of specialty chemicals, such as **liquid crystals** for displays, **effect pigments** for coatings and cosmetics, or **high-tech materials** for the electronics industry.

Product portfolio
EMD Serono has experience in perfusion and fed-batch

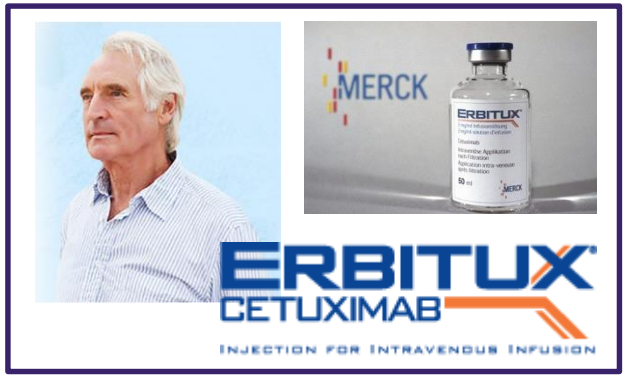
During the 90s
Perfusion processes for low productive processes and labile molecules (using different types of carriers)

Past decade
Fed-batch processes for stable proteins and high productive processes (mAbs)

Infertility

Neurology

Oncology



Growth & metabolism

Fed-batch process
Platform available including proprietary chemically defined media

Lab scale process development

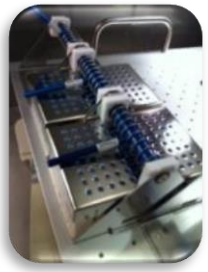
Manufacturing

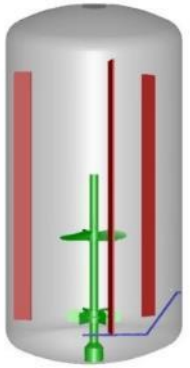
96-deepwell plates

Shake tubes

Micro-scale bioreactors

Lab-scale bioreactors





Orbital shaking

Stirred tank bioreactors

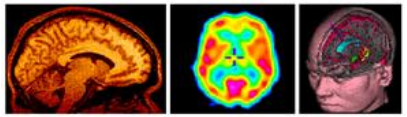
Throughput

Volume

Perfusion process
We are not going back to our perfusion processes of the 90s

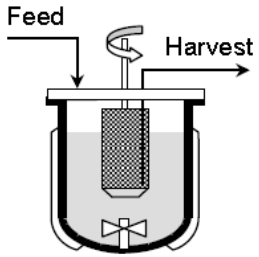
Attached cells

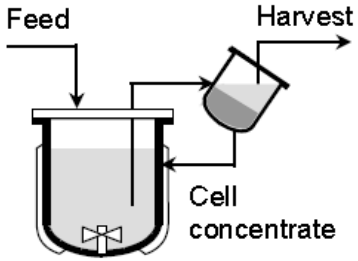
Fixed-bed

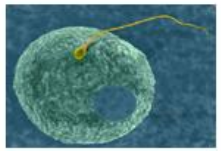


NEW FORMULATION Rebif®

µ-carriers



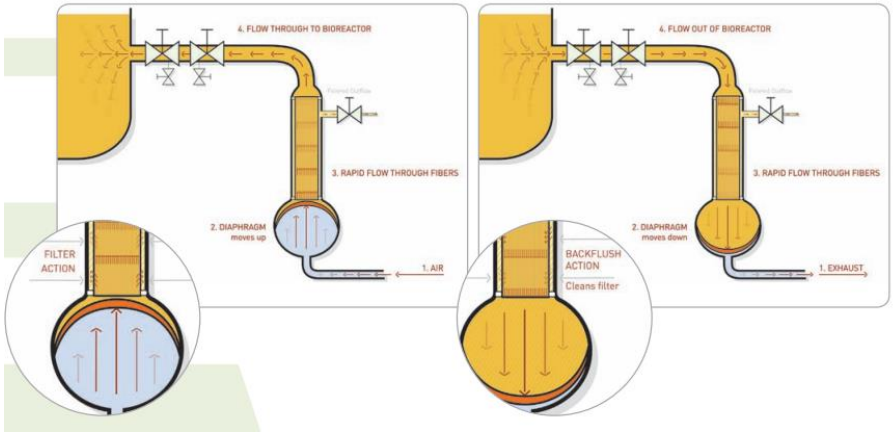




GONAL-f® FOLLITROPIN ALFA

Cells in suspension

Alternating tangential flow filtration



Perfusion process development supported by our expertise in cell culture and media development

Perfusion process development tools
Limited scale and throughput range for perfusion development

Lab scale process development

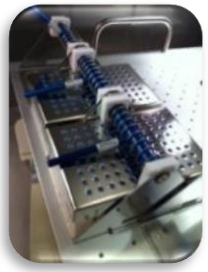
Manufacturing

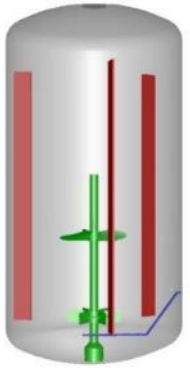
96-deepwell plates

Shake tubes

Micro-scale bioreactors

Lab-scale bioreactors





Orbital shaking

Stirred tank bioreactors

?

OK

01

FIRST STEPS IN PERFUSION PROCESS DEVELOPMENT

First steps
Evaluate perfusion technology

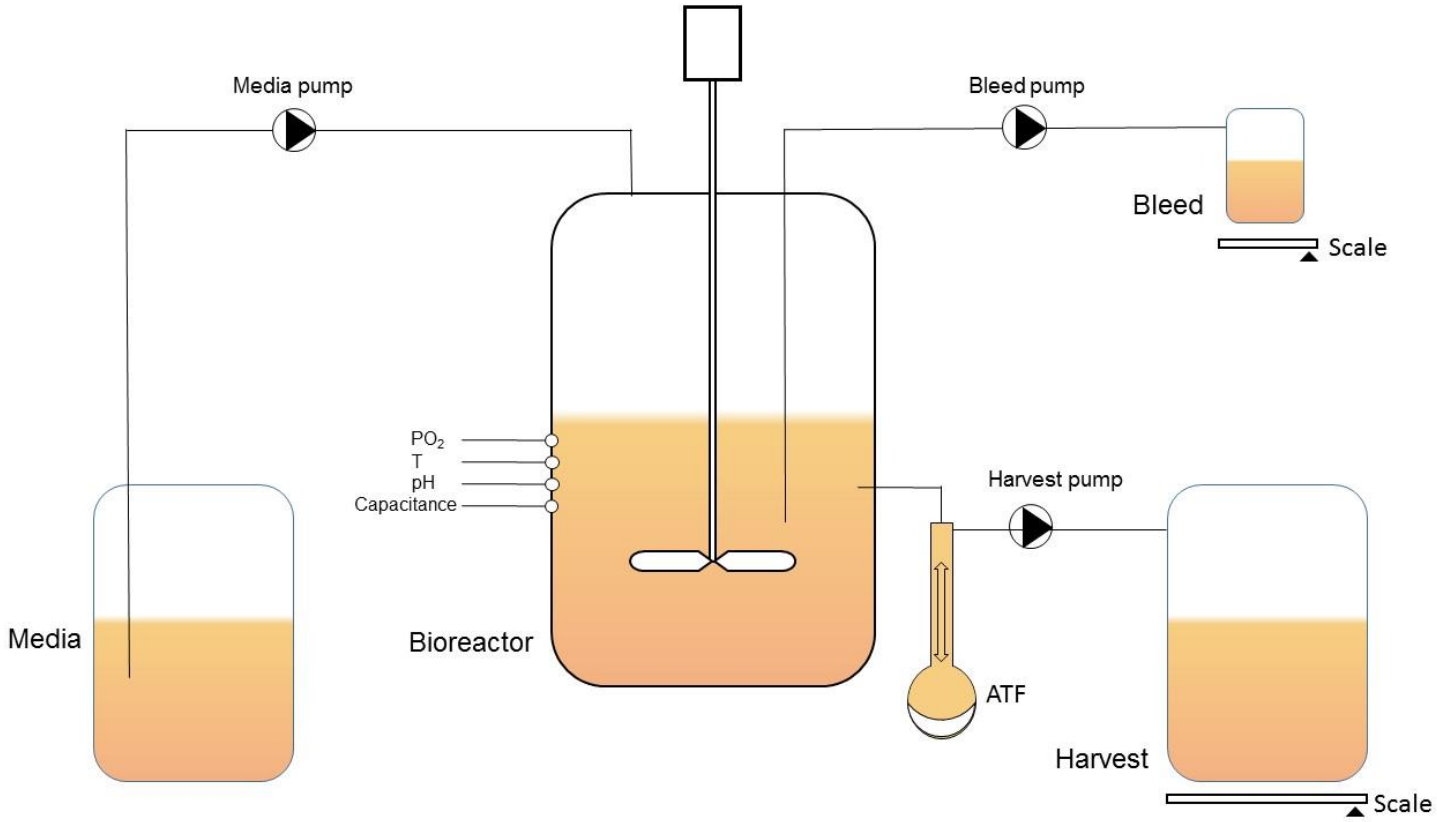
TARGET

- 1 g/L_{bio}/day protein
- 1 wvd^{-1}

STRATEGY

- Platform media
- Maintain steady-state
- Media enrichment (↑ VCD, ↓ CSPR)
- Temperature
- O_2 (↑ k_La, ↓ foam, scale-up)
- Test different cell lines

Learning the hard way
Lab set-up for 2 bioreactors



- ATF
- Continuous bleed
- Capacitance probe to monitor biomass
- Scale control based on calibration
- Lack of flowrate control…

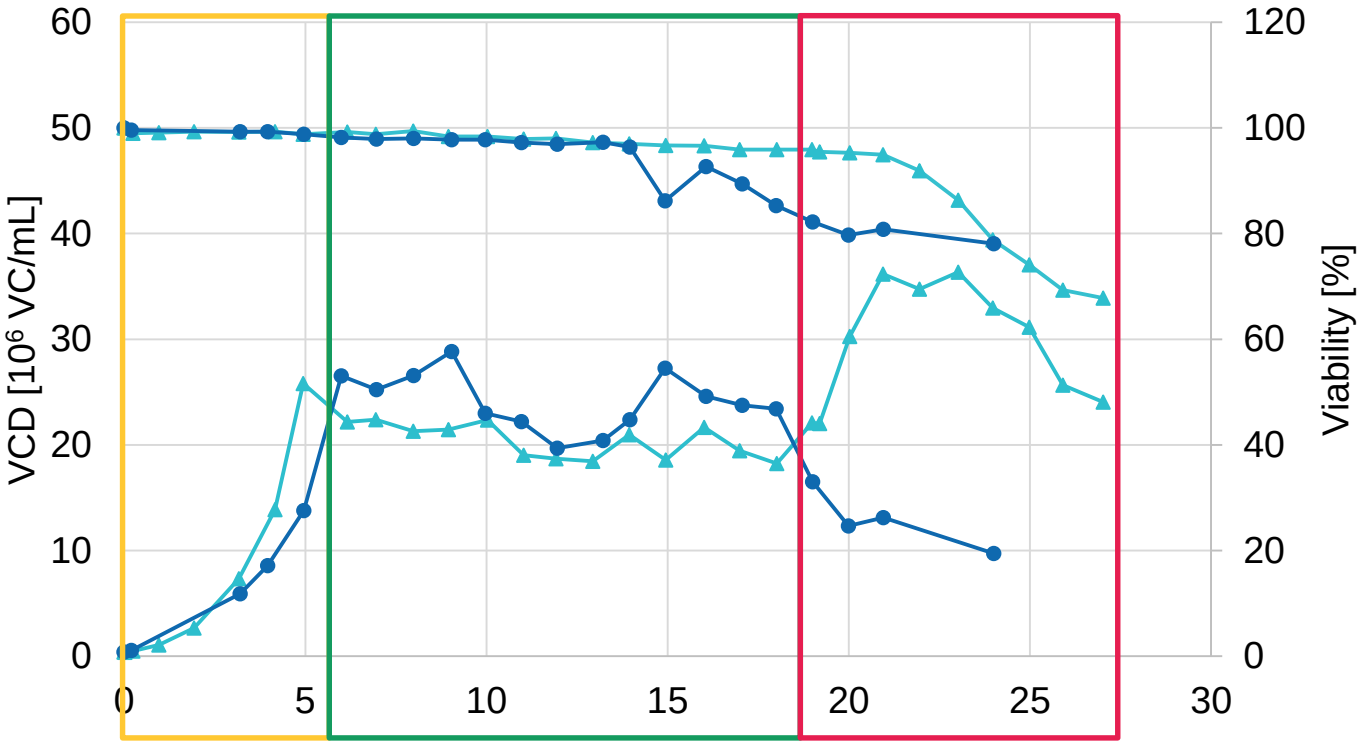
02

A FEW CASE STUDIES

Case studies

1. Easy-switch
2. Increasing perfusion rate
3. Temperature switch
4. Using perfusion rate and temperature
5. Thoughts about VCD control
6. Media enrichment

1. Easy switch
Fed-batch platform media for perfusion



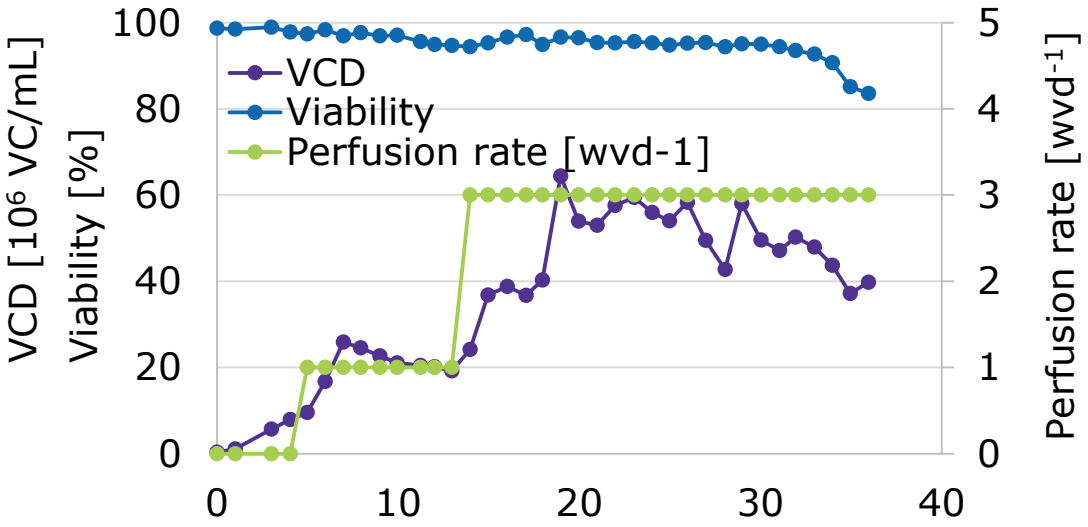
Growth phase

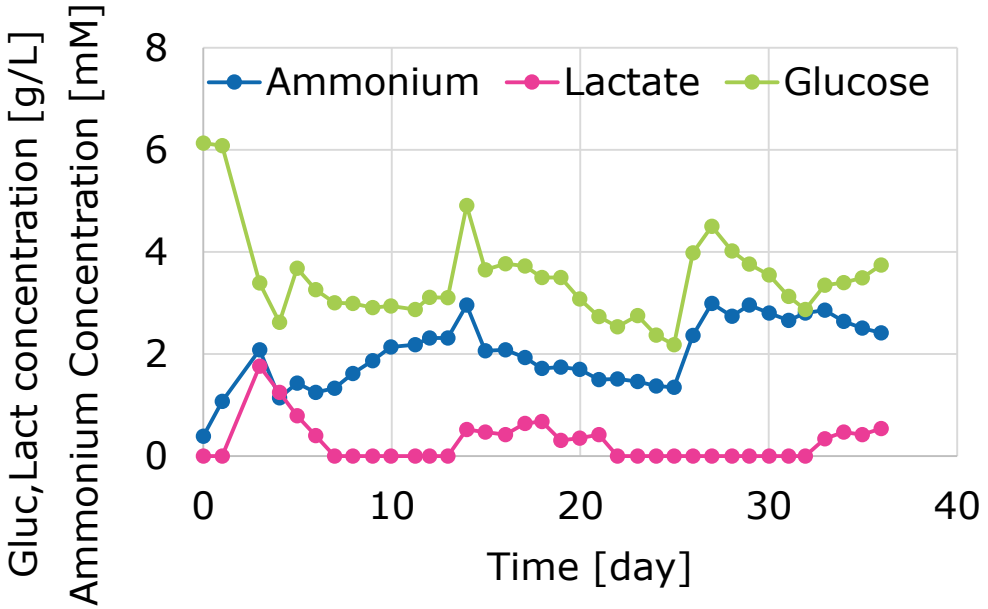
Steady-state, media supports about 20 millions cells per mL at 1 wvd^{-1}

Change conditions to see effect on steady-state to test different conditions in a single run
Bleed stopped and use of enriched media
Temperature switch

Perfusion rate used = 1 wvd^{-1}
Fixed bleed based on preliminary observations

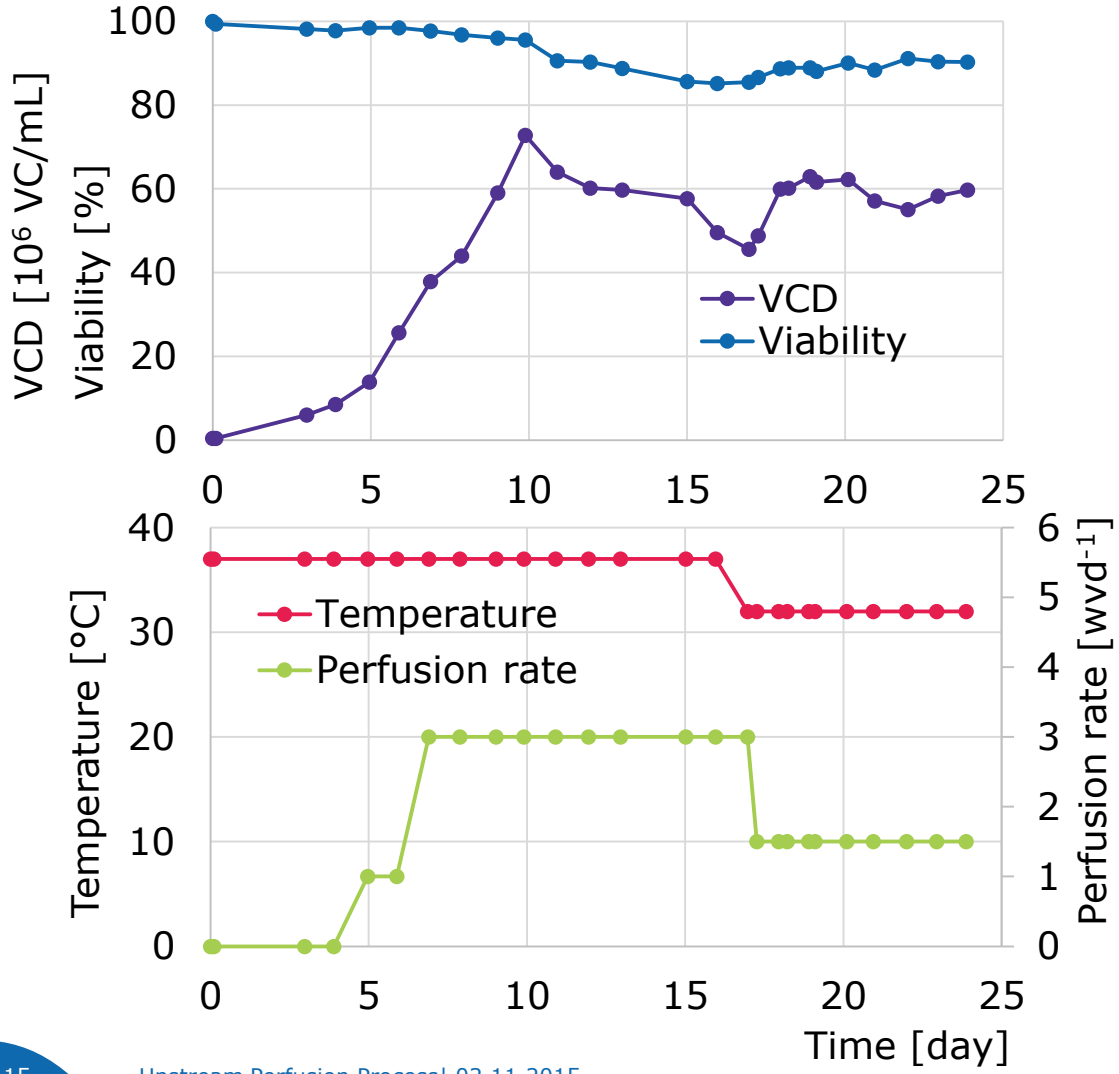
2. Increasing perfusion rate
No lactate or ammonium accumulation

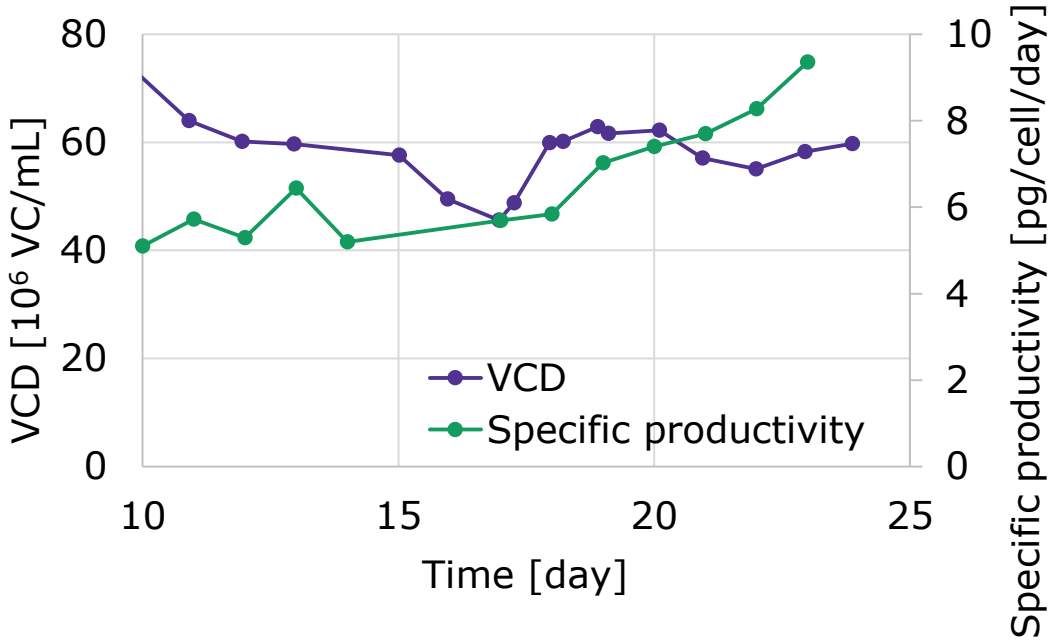




↑ perfusion rate = ↑ VCD
Bleed rate adapted for each condition (20 → 60 → T shift)
T shift at day 25 → bleed stopped
No amino acid depletion measured

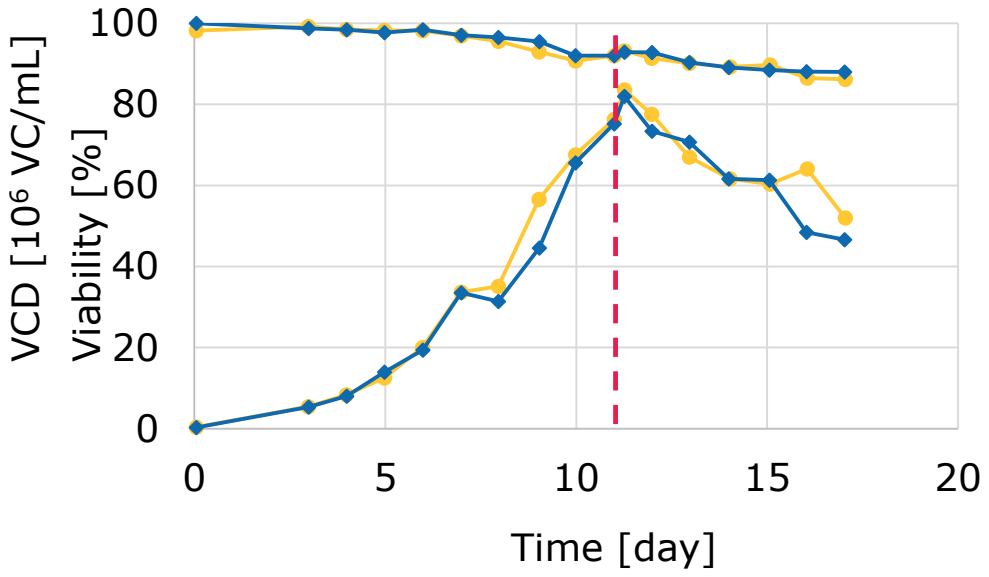
3. Temperature switch
Reduce perfusion rate and increase productivity

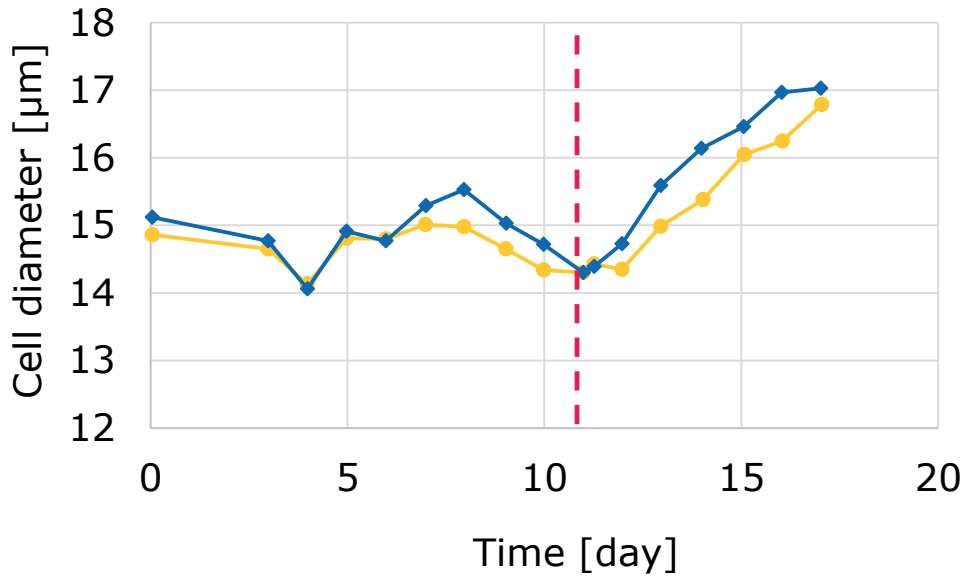




↓ Temperature
↓ Perfusion rate (CSPR from about 50 to 25 pL/cell/day)
↑ Specific productivity

4. Using perfusion rate and temperature
Switch from growth phase to steady-state is sensitive





Temperature Switch
Perfusion rate decreased

VCD drop when growth phase is stopped (recurrent issue)
No glucose or amino acid depletion observed

Cell metabolism changes

5. Thoughts about VCD control
Self maintaining steady-state or feedback loop?

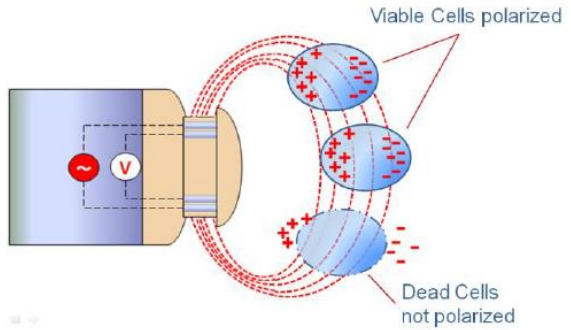
1. Can we reach steady-state without a feedback loop on VCD?

   No feedback control loop
   Fixed bleed and perfusion rate

$$\mu_{growth} = \mu_{death} + \mu_{bleed}$$

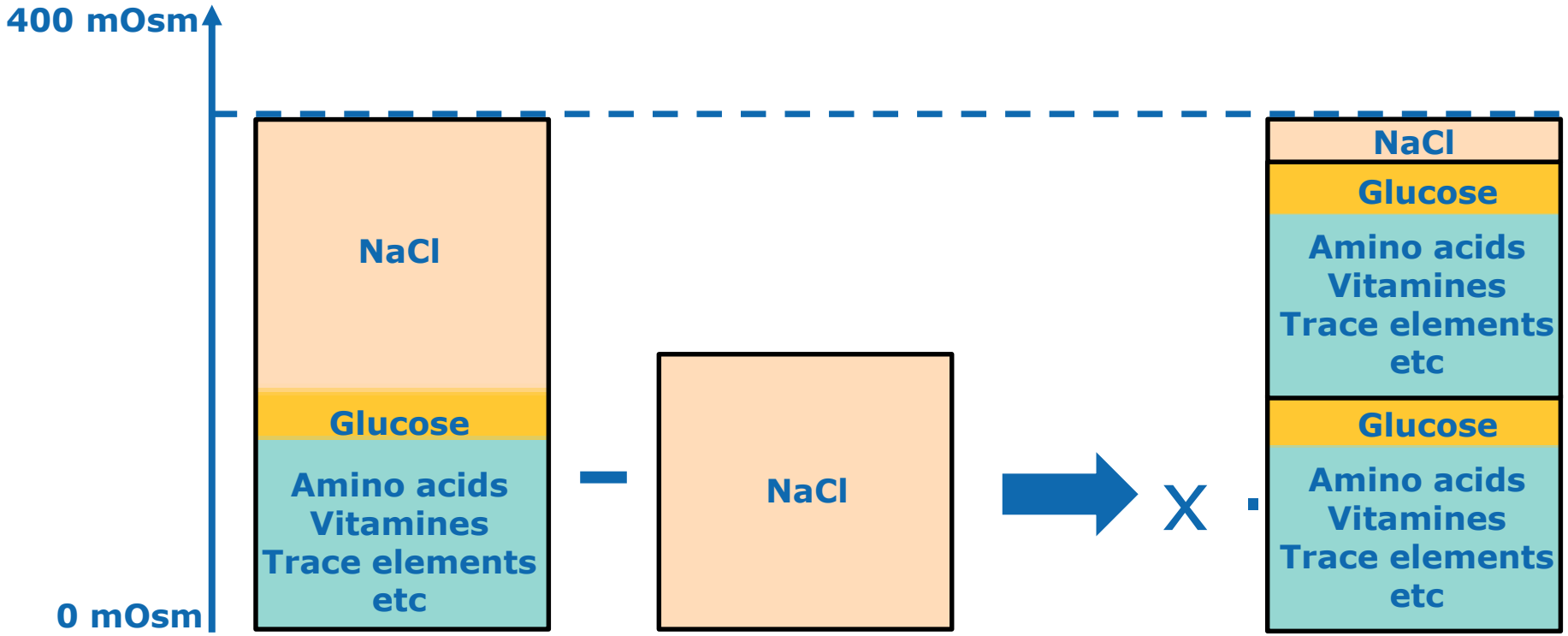
2. Do we need a signal to monitor and control the bleed online?

   Feedback control loop for biomass
   Bleed varies depending on signal



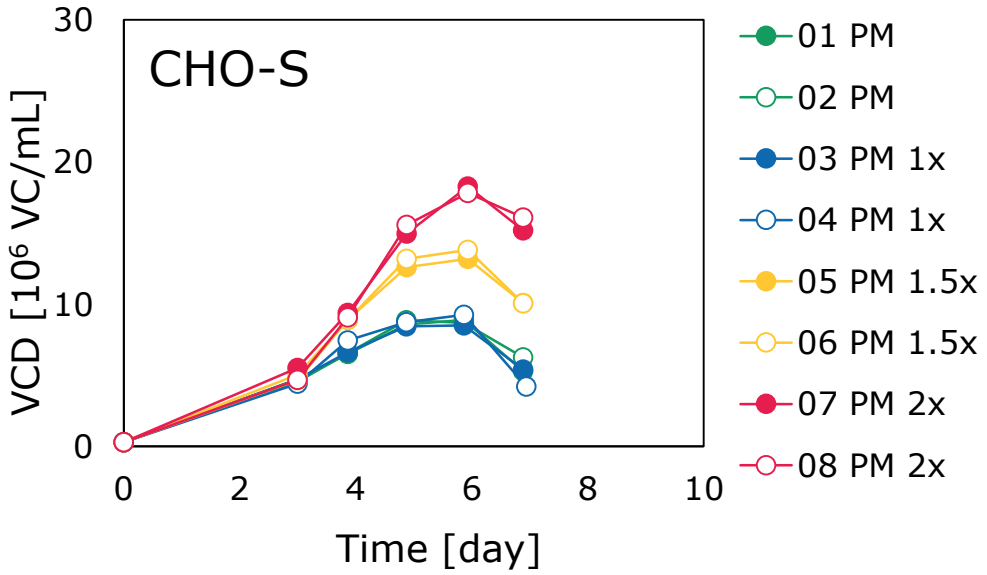
http://cercell.com/media/1460/fogale-capacitance-how-does-it-work.pdf

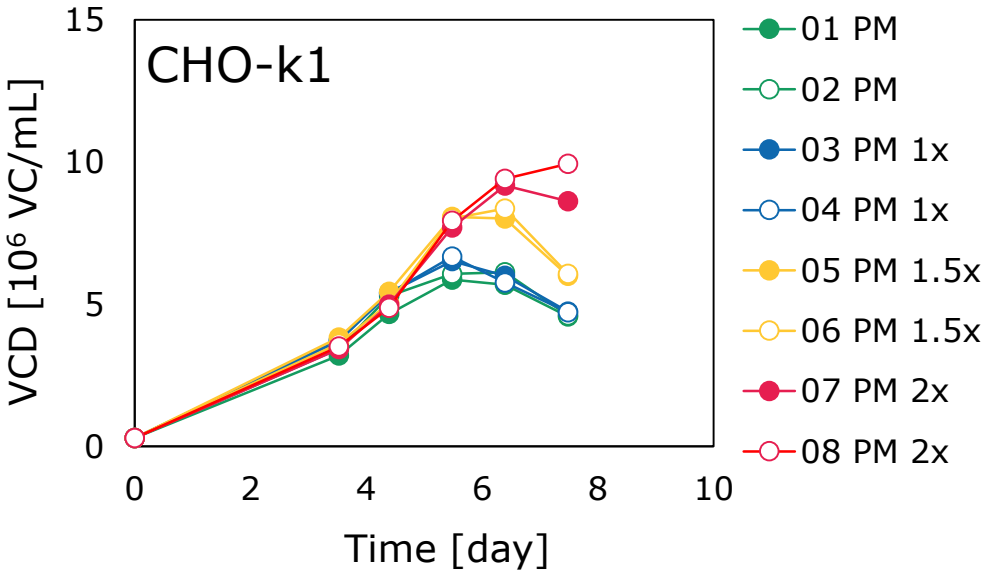
6. Media enrichment
Concentrate media without affecting compound balance



6. Media enrichment
Batch evaluation of NaCl depleted formulation at different concentrations

PM = Platform media with original powder formulation
PM #x = Platform media with NaCl depleted powder concentrated # times





04 CONCLUSION

Conclusion

- Perfusion with the platform media and 20 million cells per mL at 1 wvd^{-1}
- Increased perfusion rate to explore steady-states at about 60 million cells per mL
- Observed effect of temperature change
- Media concentration strategy tests in batch mode encouraging

Next Steps

- Volume control implementation
- VCD control strategy needs to be defined
- Increase perfusion capacity in our development lab

Acknowledgments

Head of BPS
Hervé Broly

BioTechnology & Innovation Team
Luis Ayala
David Brühlmann
Xavier LeSaout
Jonathan Souquet

BPS-USP team, especially
Martin Jordan
Matthieu Stettler
Lorette Petitperrin
Thomas Solacroup
Milène Marsaut

BPS-Analytics team