

DTU



Assoc. Prof. Ulrich Krühne

Dept. of Chemical and Biochemical Engineering
Technical University of Denmark

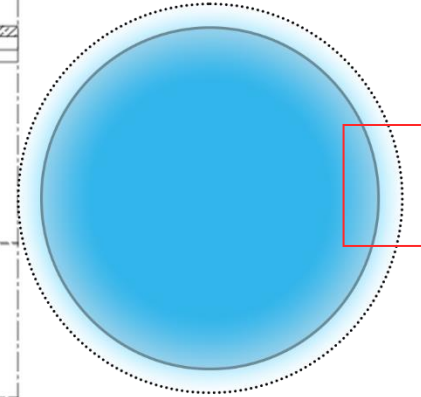
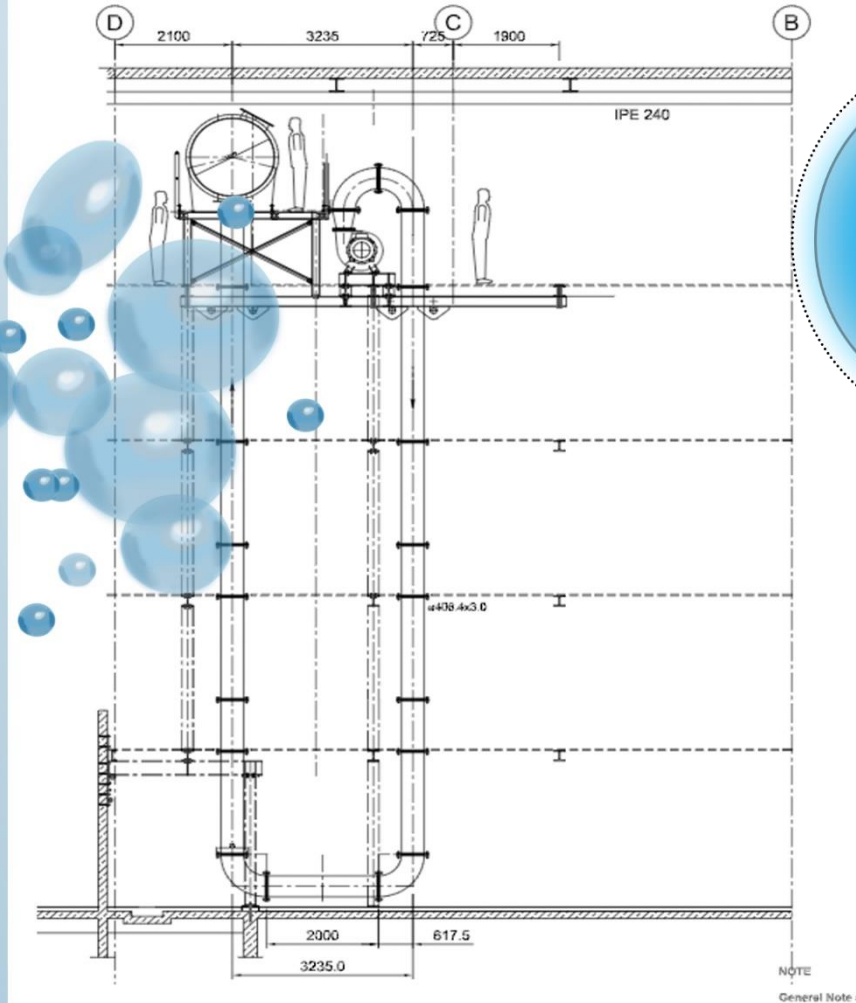
From Micro to Macro – Scale Matters

Introduction

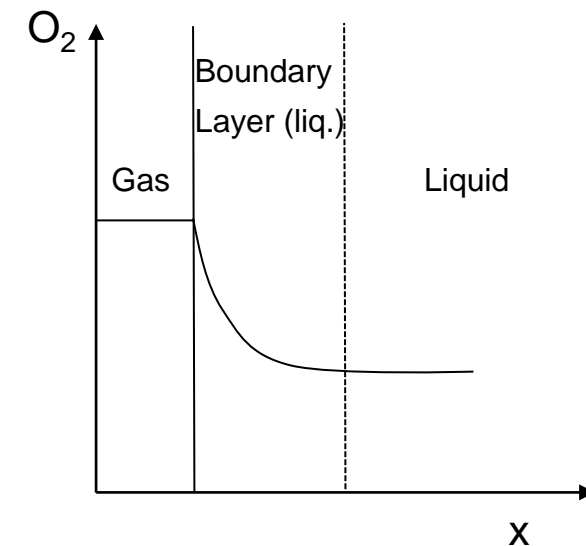
- Safety / Programme
- Some basics about Bioprocesses
 - Microorganism
 - Mass transfer
 - Gradients
 - Mixing
- CFD – Does it work?
- Microscale
 - μ bubble reactor
- Pilot Scale
 - Endoscopic Measurements
 - U-Loop Reactor (Unibio A/S)
 - Mixing Time
 - FBA
- Full Scale
 - What if scenarios
 - Compartmentalization
 - Automatic/Simple
- Membranes
 - Dynamic Filtration
 - SPTFF
- The Future is
 - Scale Down
 - Dynamic
 - Networks

➔ 1 mm = 4.16 min
3.16 mm = 41.66 min

Bioprocesses



$$\dot{m} = k_L a \cdot (C_{O_2, sat} - C_{O_2})$$



My life in the bioreactor (seen from the bug life)

tracer

recirculation behind
the cooling coil

high glucose
low oxygen

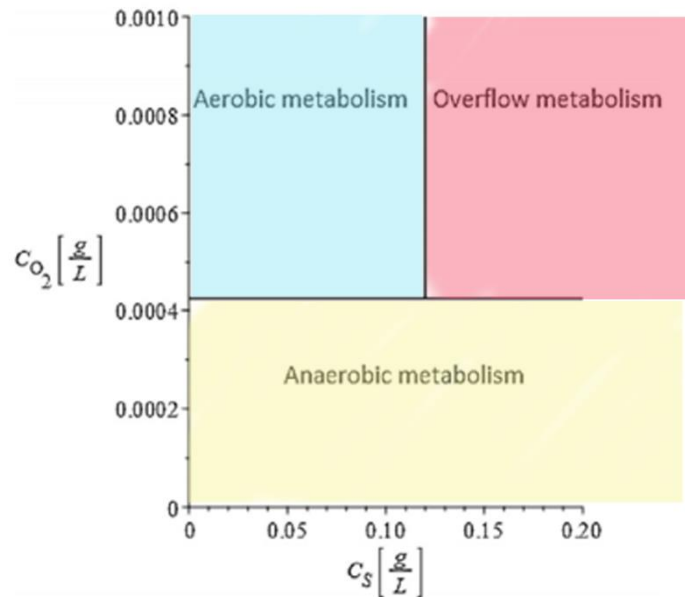
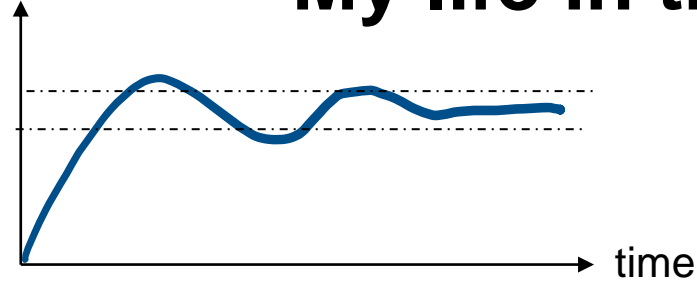
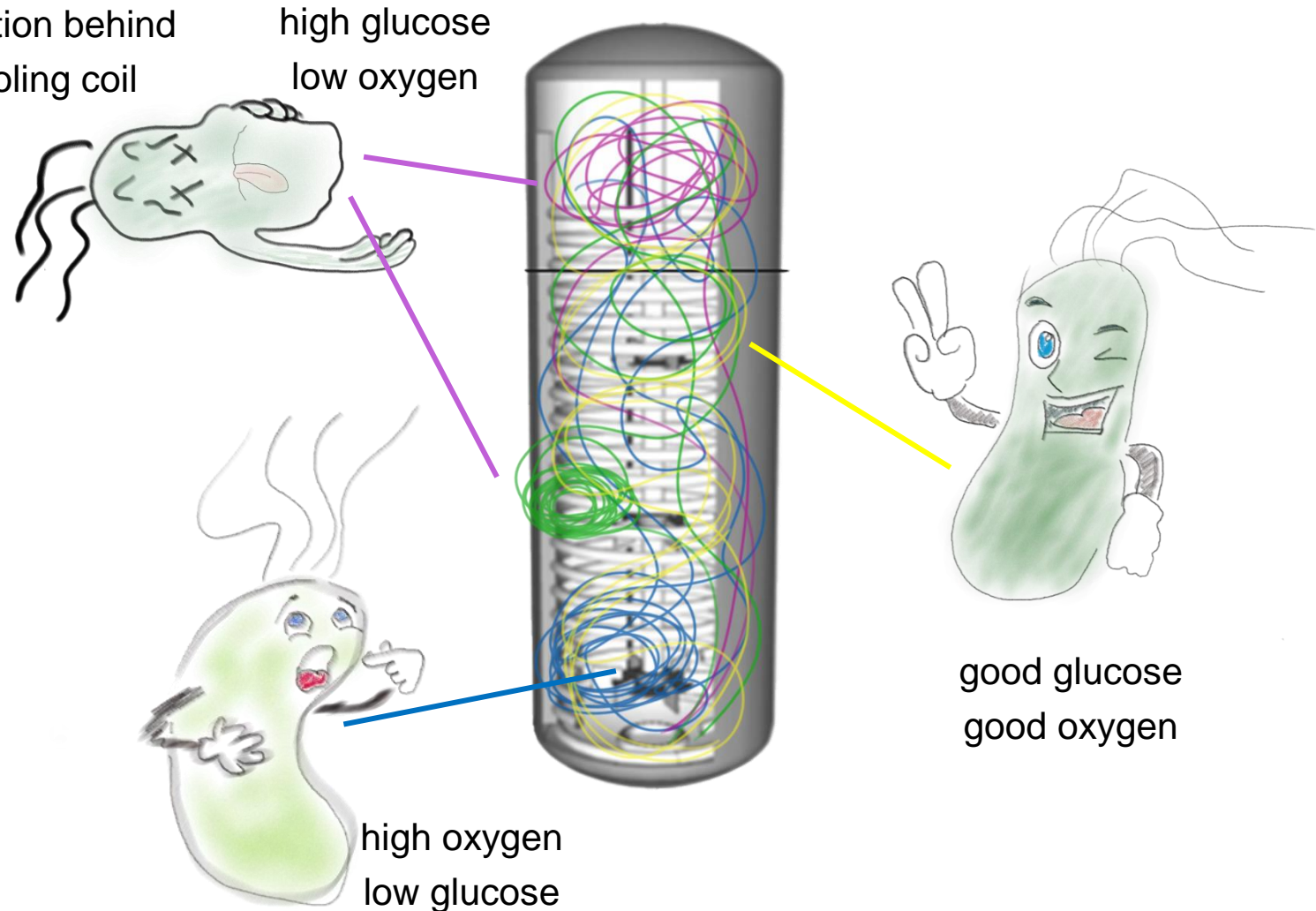
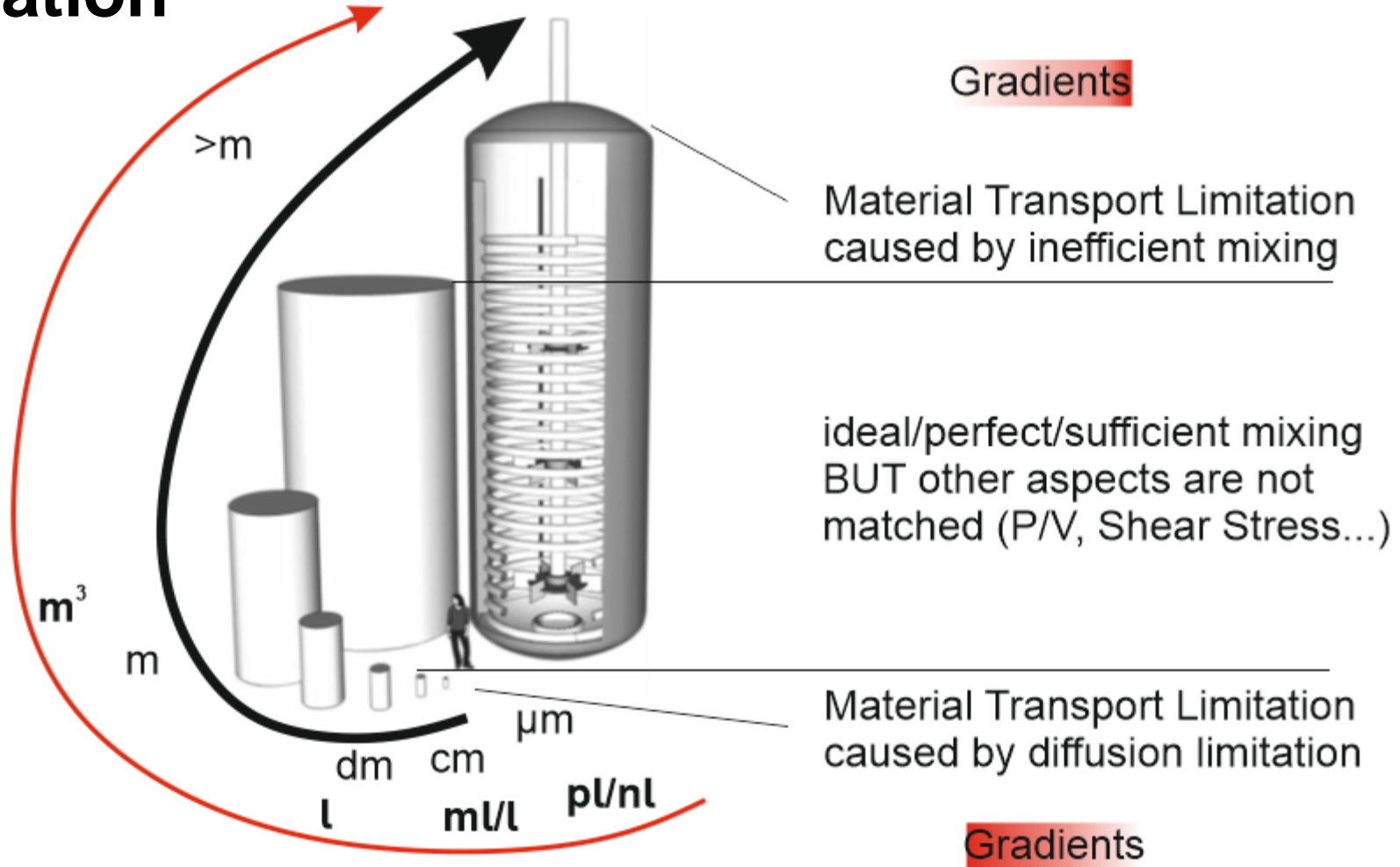
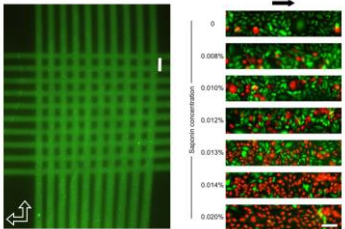
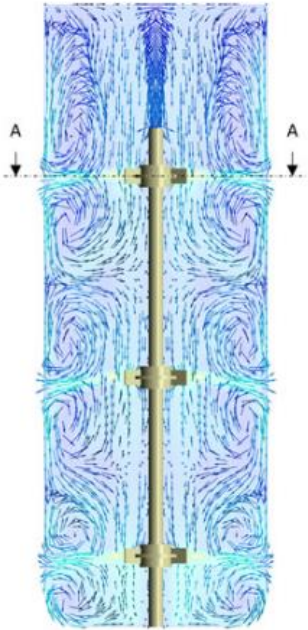


Fig. 3 – Boundaries between aerobic metabolism, overflow metabolism, and anaerobic metabolism defined in the kinetic model of *S. cerevisiae*.

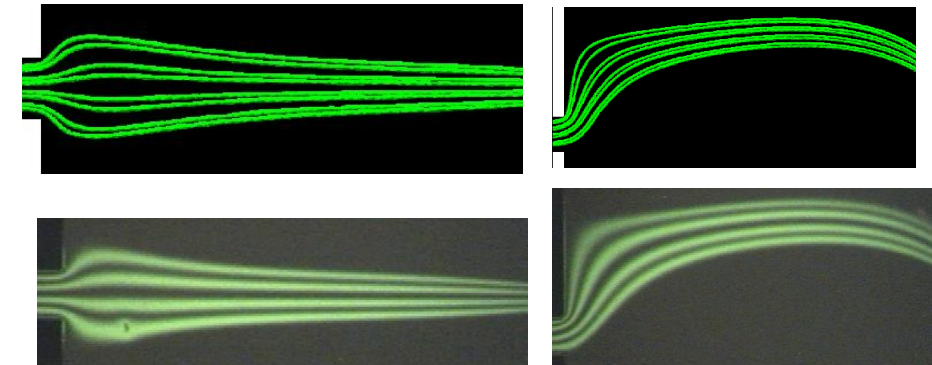
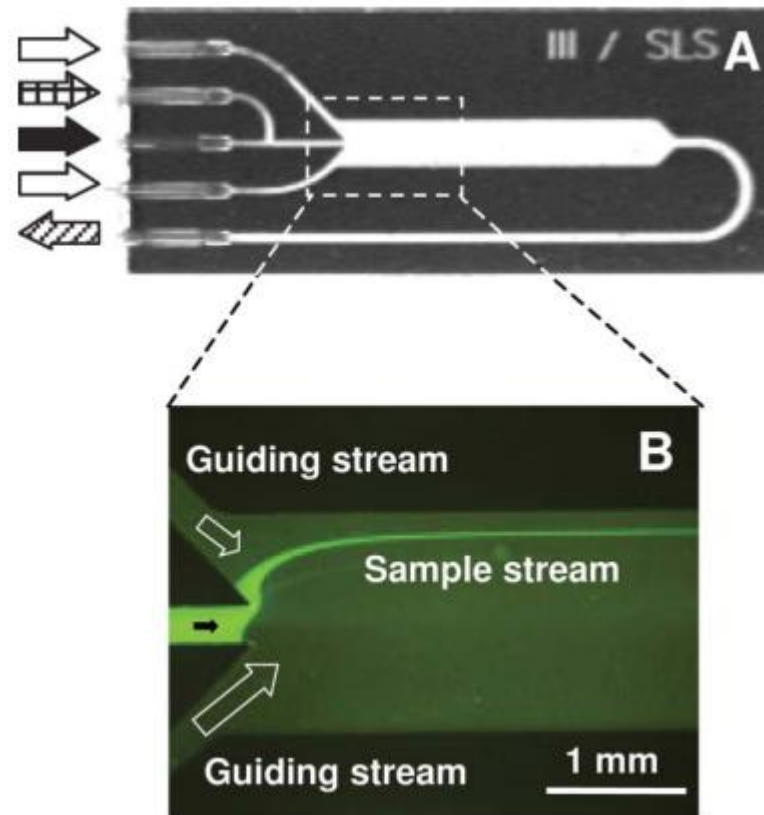
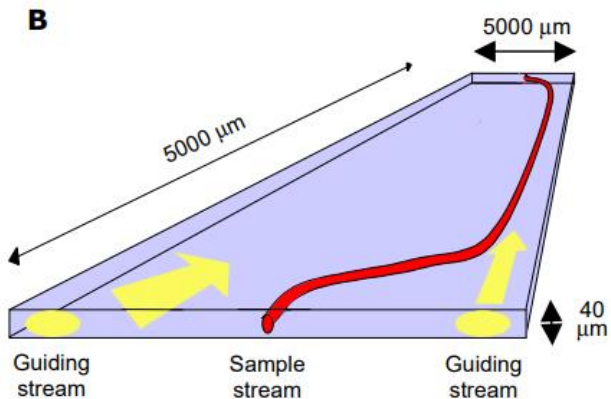
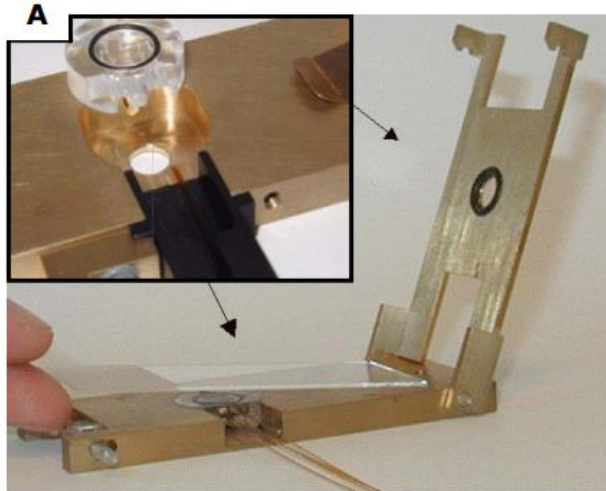


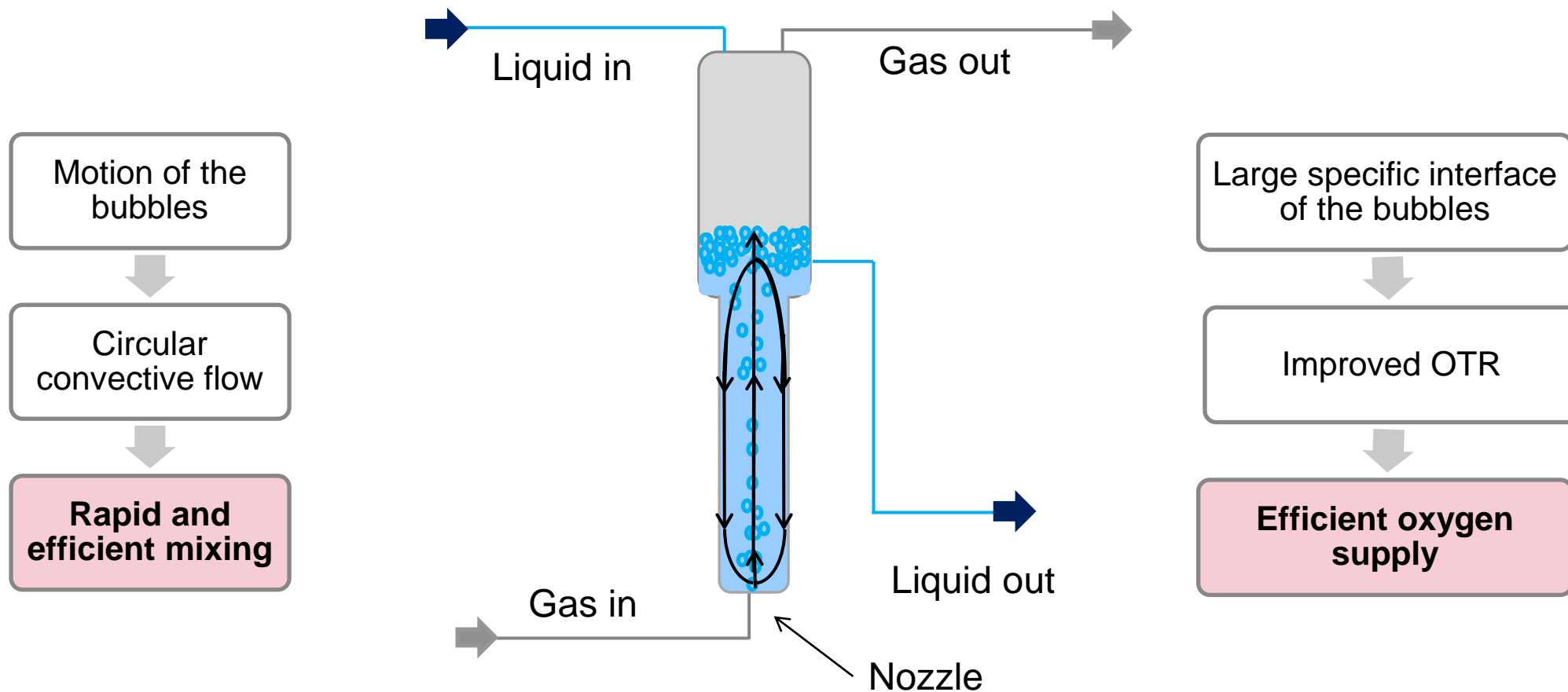
Motivation



Stocks, S.M., 2013. Industrial enzyme production for the food and beverage industries?: process scale up and scale down, in: Microbial Production of Food Ingredients, Enzymes and Nutraceuticals.

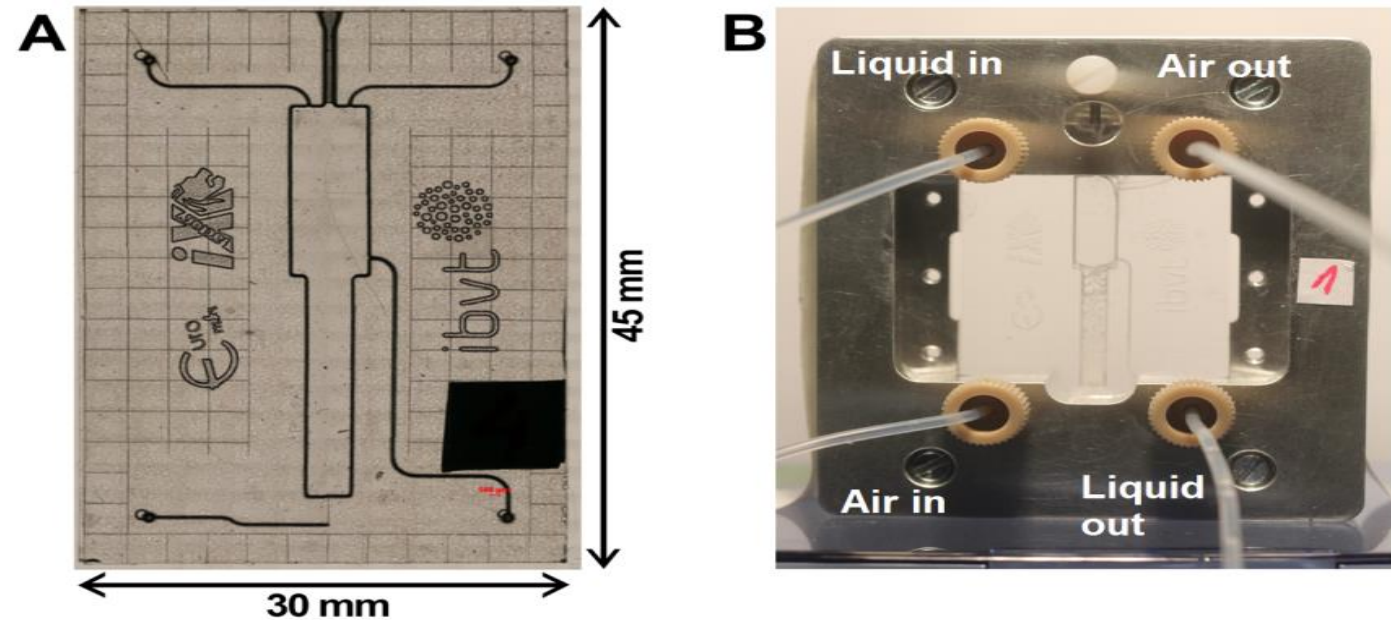
Does it work?



**Bubble column reactors + Microbioreactor =
Microbubble column-bioreactor (μBC)**

Glass microbubble column-bioreactor ($g\mu BC$)

Properties	
Material	Borosilicate glass
Technology	Powder blasting / wet etching
Reactor volume	60 μL
Dimensions (width, height, depth)	3 mm x 19 mm x 1 mm
Nozzle	26 μm
Optical density	Miniature spectrometer, Ocean Optics
Dissolved oxygen	Microneedle sensor, Pyroscience



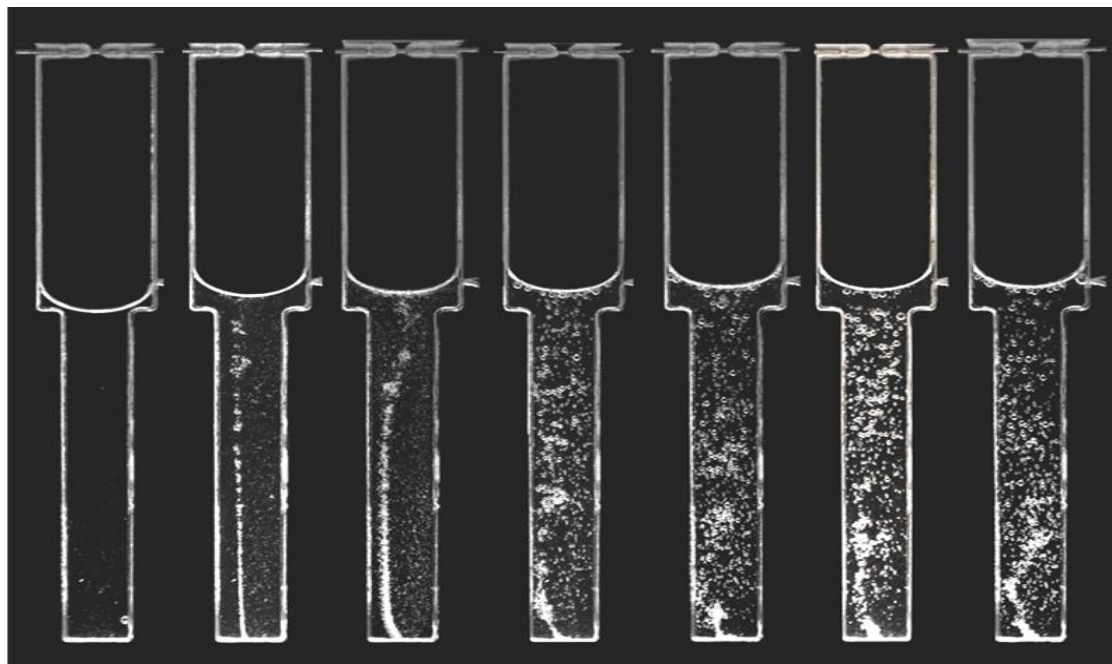
Lladó Maldonado, Rasch D, Kasjanow A, Bouwes, D., Krühne, U., Krull, R. (2018) Biochem Eng J 139:57–67

Mixing performance of the $g\mu BC$

Airflow rate Q_G

Superficial gas velocity u_G ,

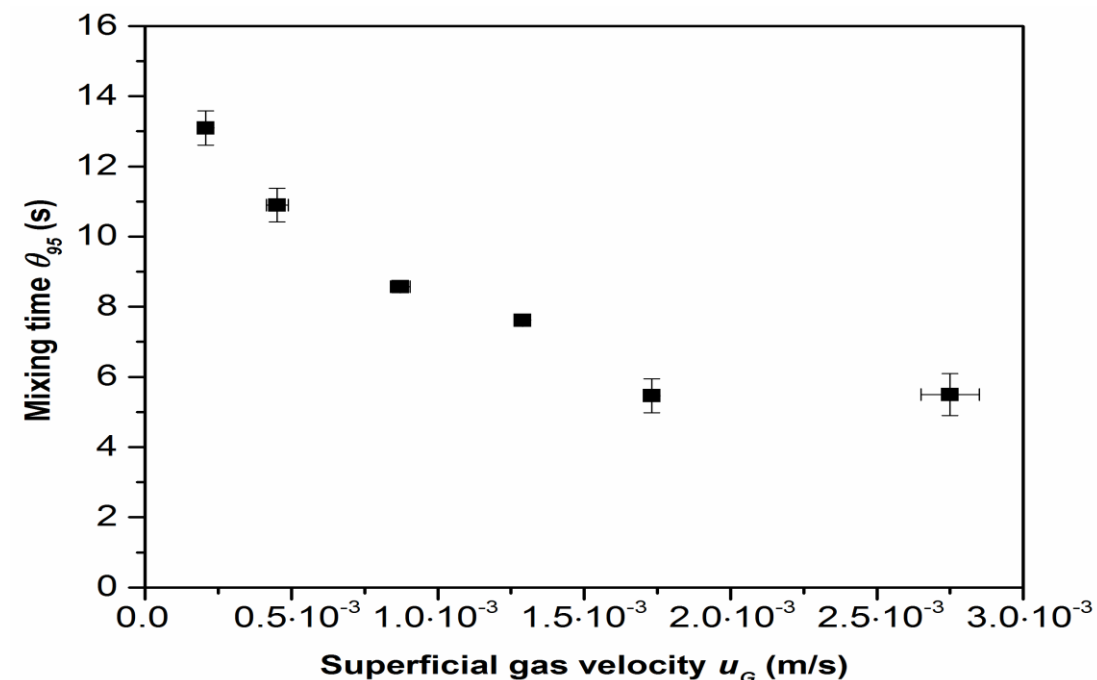
$$u_G = \frac{Q_G}{A}$$



0.0 $0.2 \cdot 10^{-3}$ $0.5 \cdot 10^{-3}$ $0.9 \cdot 10^{-3}$ $1.3 \cdot 10^{-3}$ $1.7 \cdot 10^{-3}$ $2.2 \cdot 10^{-3}$

Superficial gas velocity u_G (m/s)

Determination of the mixing time θ_{95}

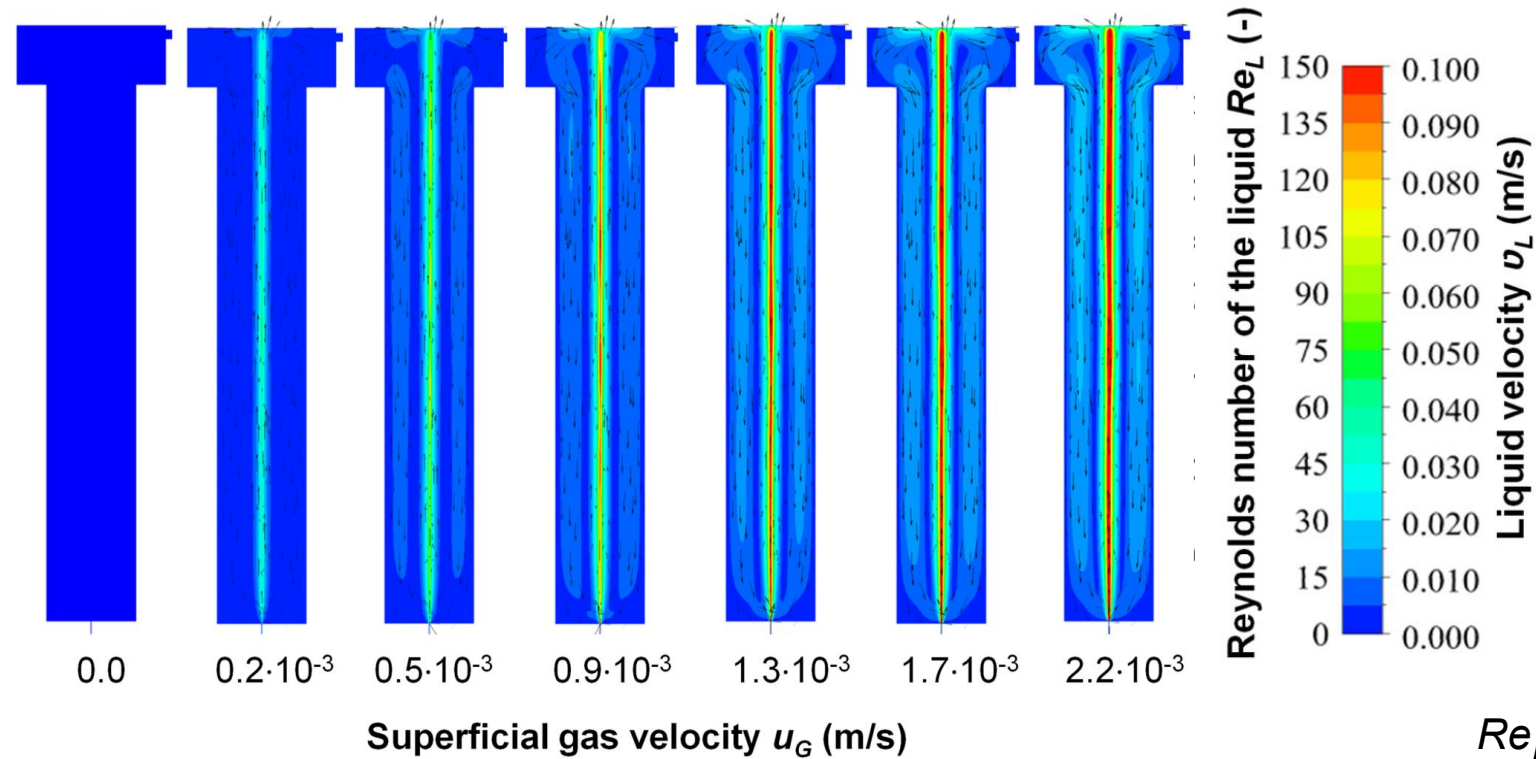


Effect of the superficial gas velocity (u_G) on the mixing time

Mixing time down to 5.5 s

Liquid velocity v_L and Reynolds number Re_L

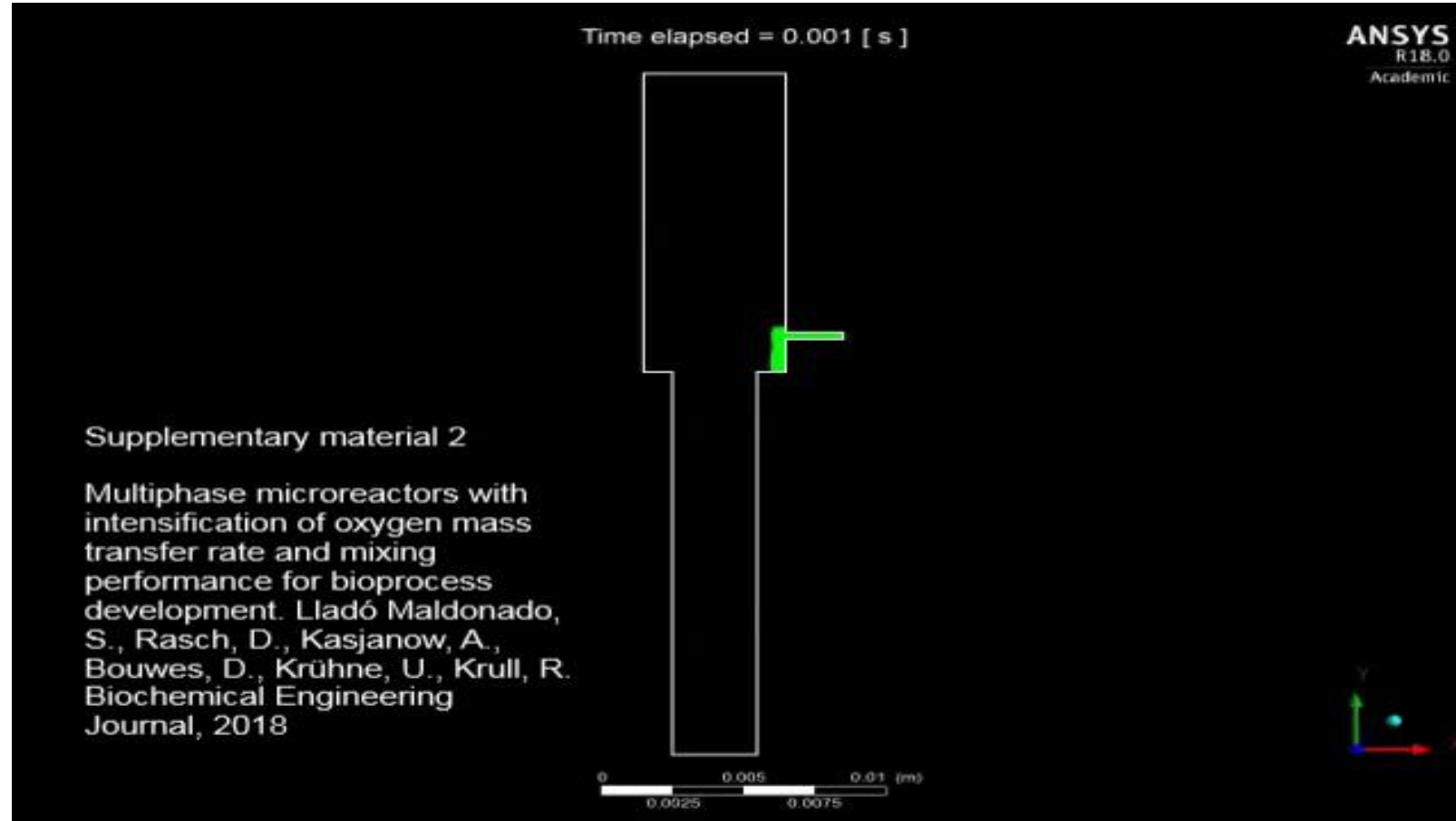
Computational Fluid Dynamic (CFD) model



Two-phase simulations to model the fluid velocity field of gas and liquid phases at different airflow rates.

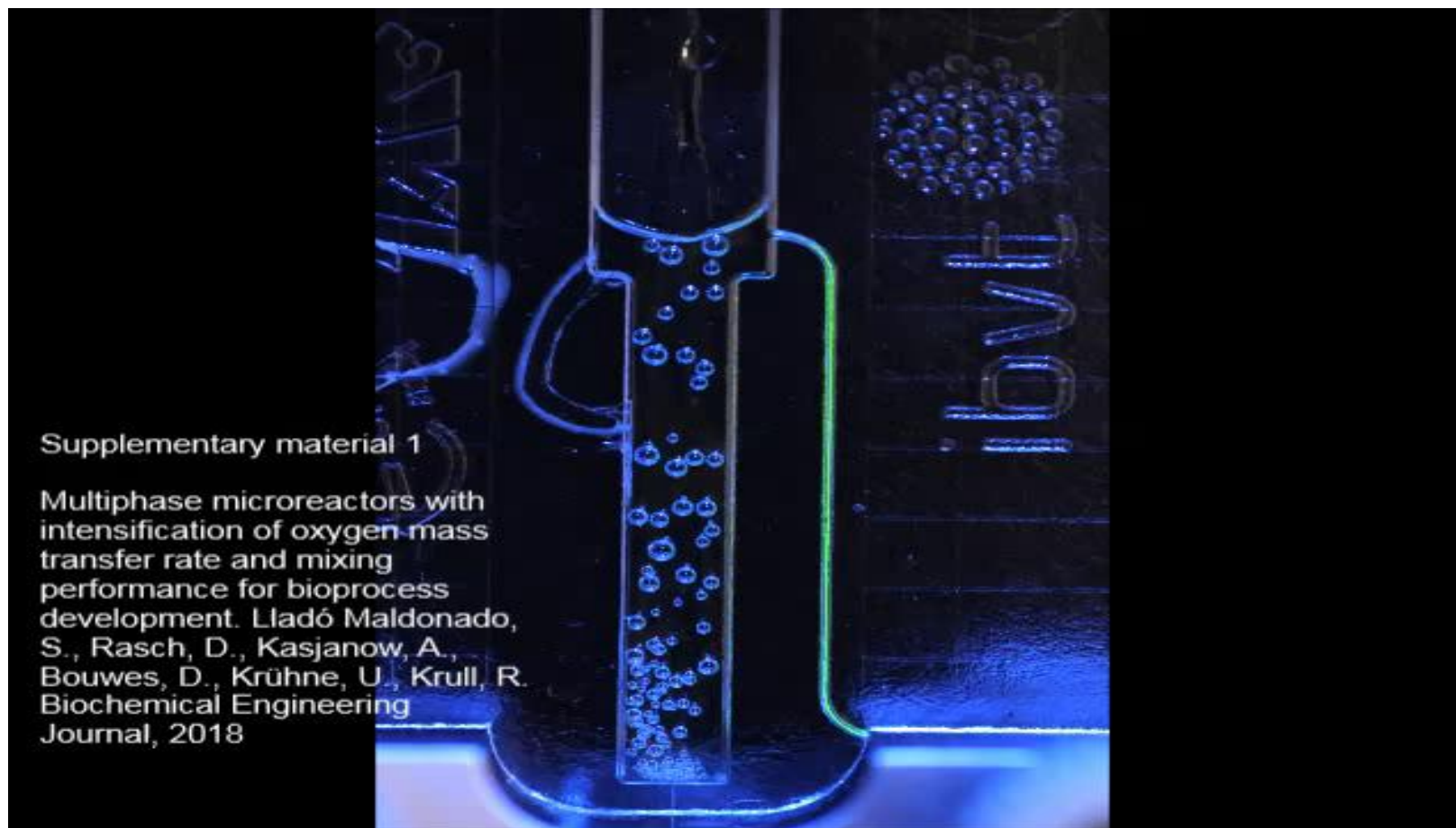
$$Re_L \equiv \frac{\rho \cdot u_L \cdot d_h}{\mu}$$

$\rho = 1000 \text{ kg/m}^3$
 $d_h = 1.5 \cdot 10^{-3} \text{ m}$
 $\mu = 0.001 \text{ kg/(m}\cdot\text{s)}$



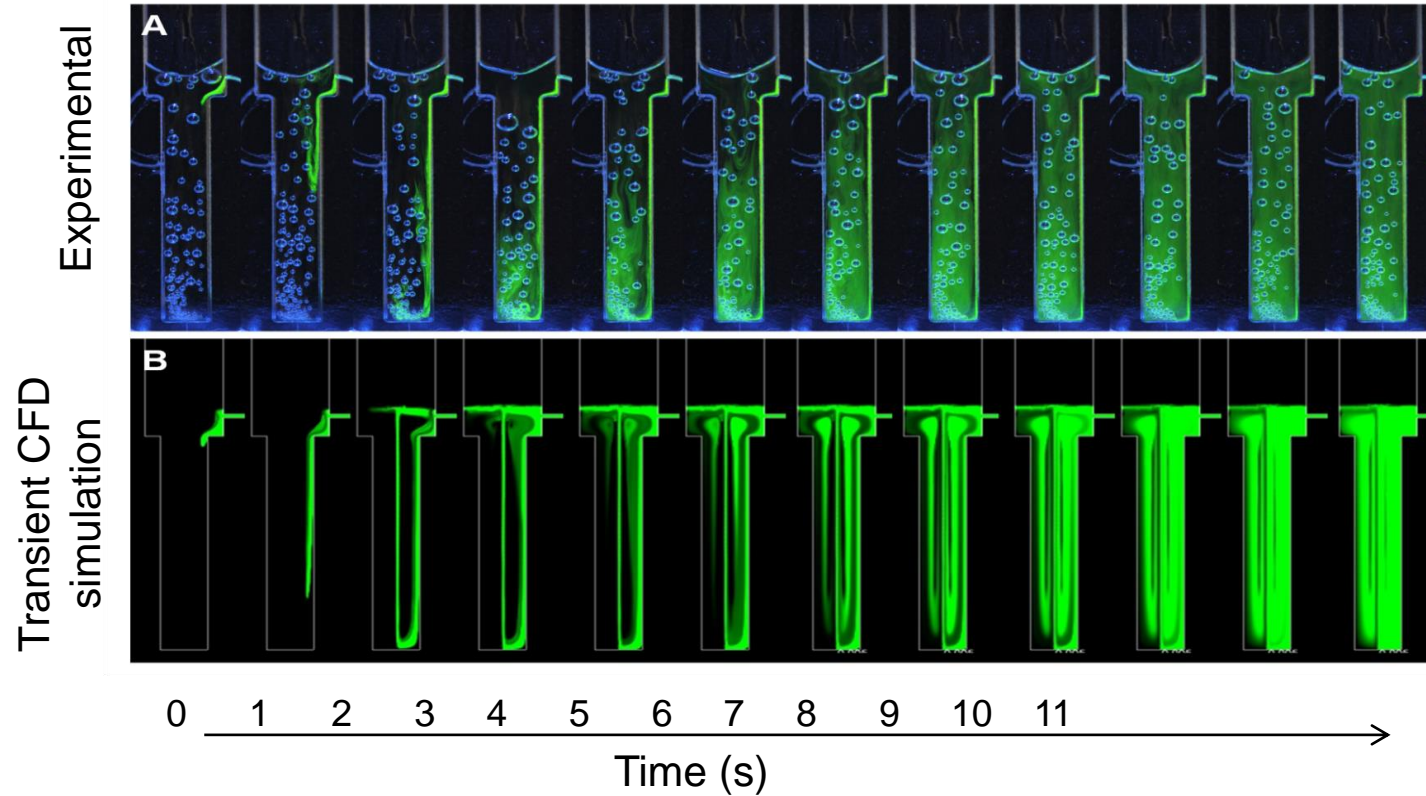
Transient CFD simulation

Mixing of a pulse of tracer at a u_G of $1.3 \cdot 10^{-3}$ m/s

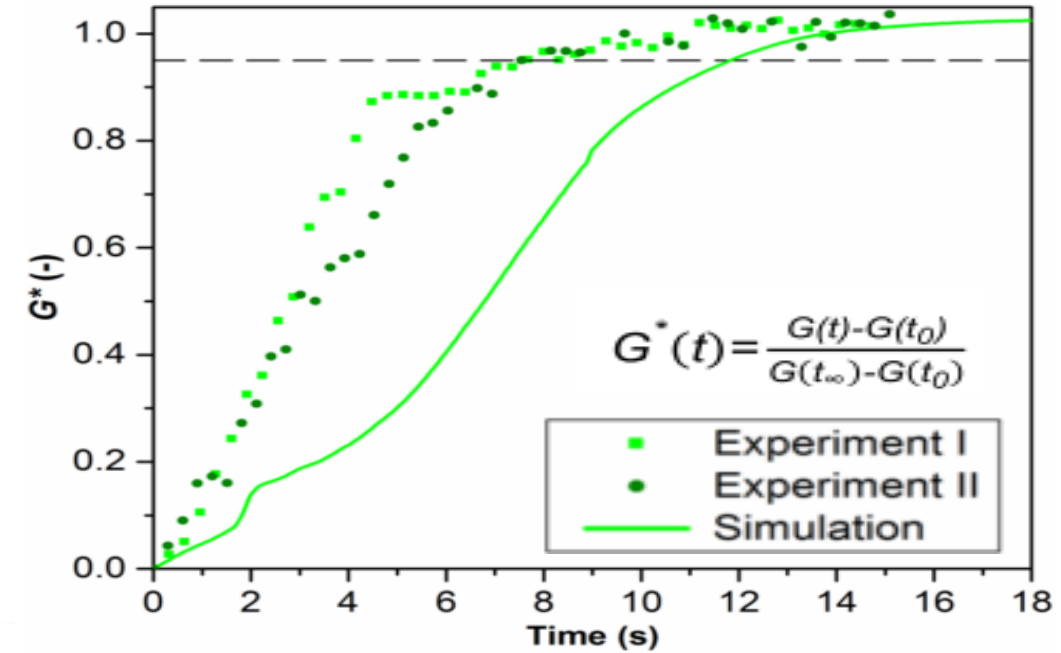


Experimental

Mixing of a pulse of 2 μL tracer at a u_G of $1.3 \cdot 10^{-3} \text{ m/s}$



Time-lapse image series after injection of 2 μ L tracer at a u_G of $1.3 \cdot 10^{-3}$ m/s



Comparison of the experimental and simulated variation of the green- channel data G^* after the tracer pulse

Scaling up (and Down)

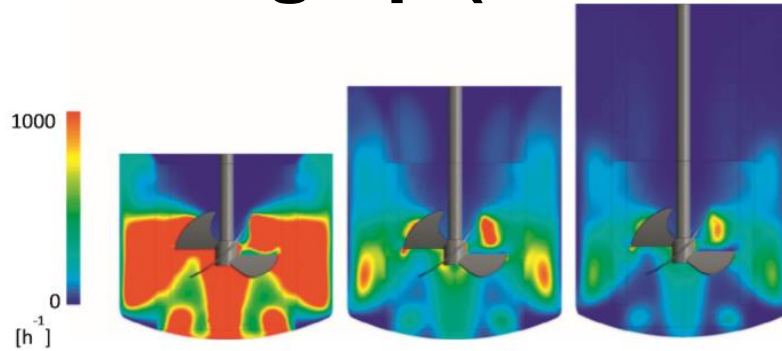


Fig. 8. Predicted distribution of $k_L a$ values for constant agitation and aeration with increasing volume and viscosity due to continuous feeding and subsequent growth.

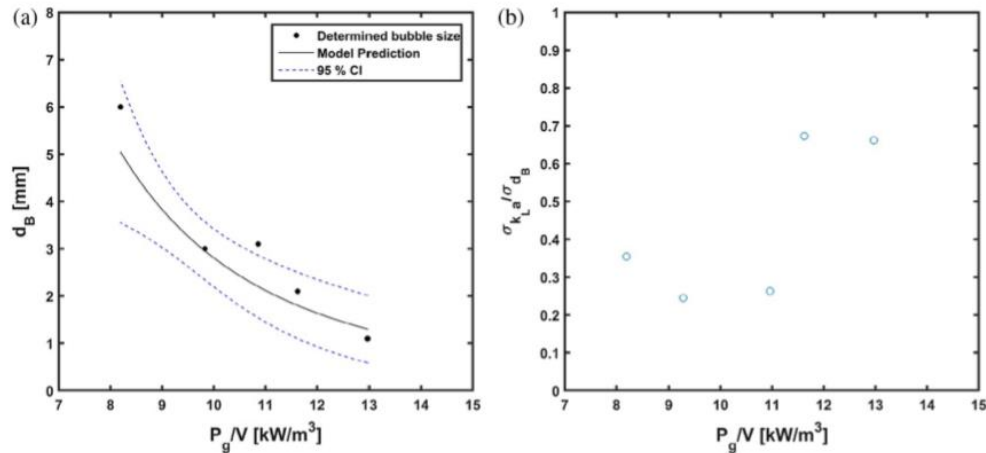


Fig. 9. Uncertainty of bubble size correlation at 200 NL/min (a) and subsequent impact on mass transfer prediction (b).

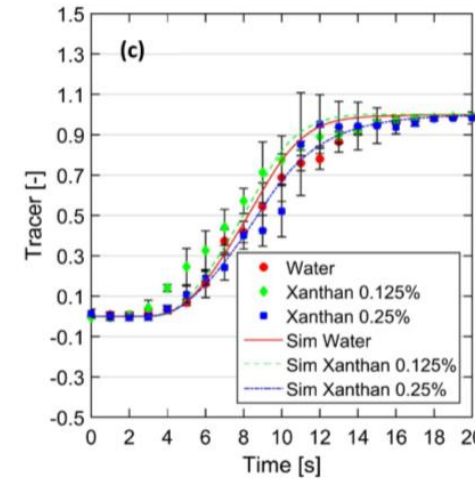
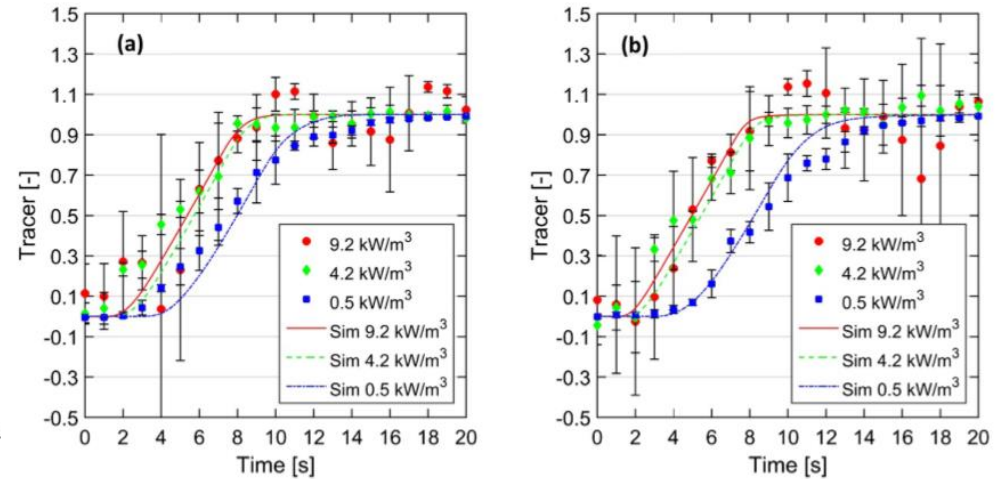


Fig. 4. Dimensionless tracer response to pulse injection for water at varying power input (a), 0.125% Xanthan at varying power input (b) and the three fluids at 0.5 kW/m³ (c). The error bars indicate one standard deviation obtained from triplicate experiments.



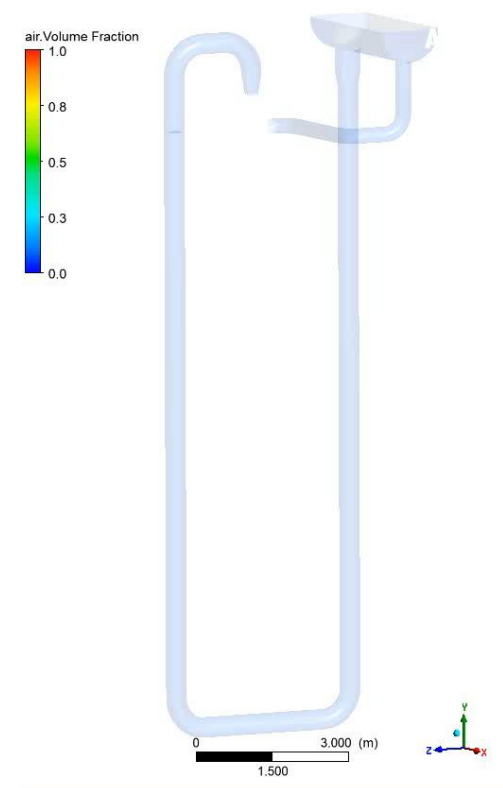
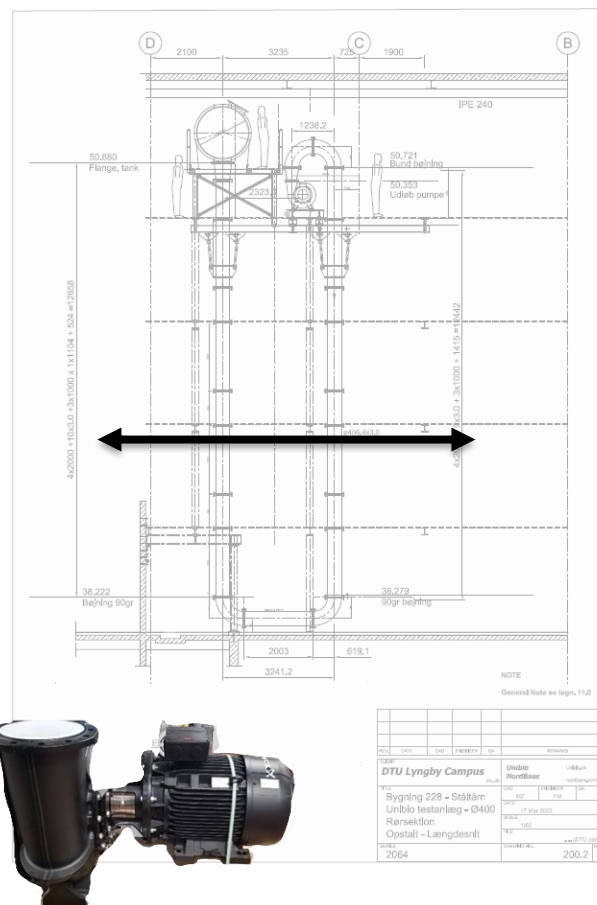
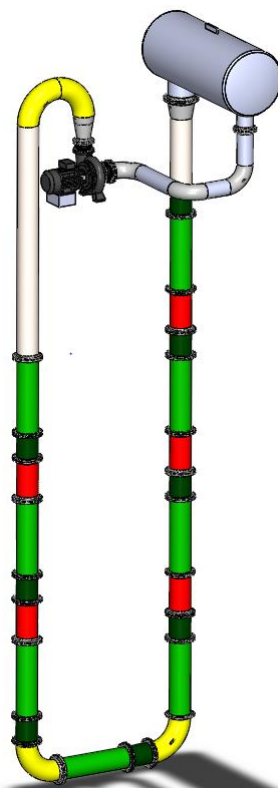
PhD Christian Bach

Christian Bach, Jifeng Yang, Hilde Larsson, Stuart M. Stocks, Krist V. Gernaey, Mads O. Albaek and Ulrich Krühne, (2017) 'Evaluation of mixing and mass transfer in a stirred pilot scale bioreactor utilizing CFD', *Chemical Engineering Science*, 171, pp. 19–26.

The Development of a Digital Twin for Mixing Observation and Optimization

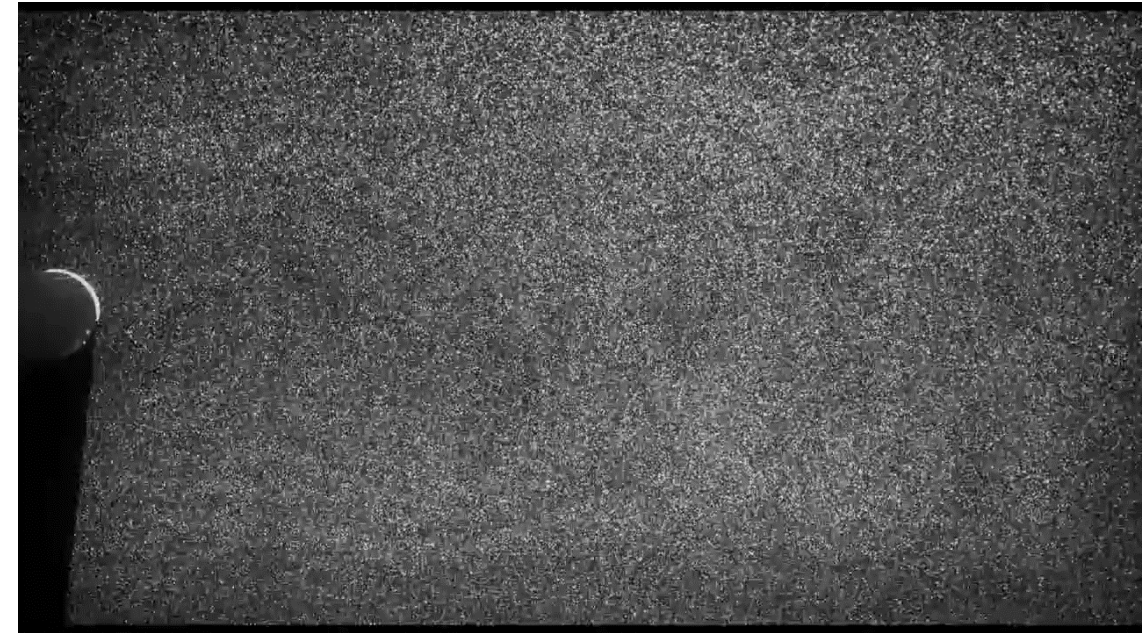
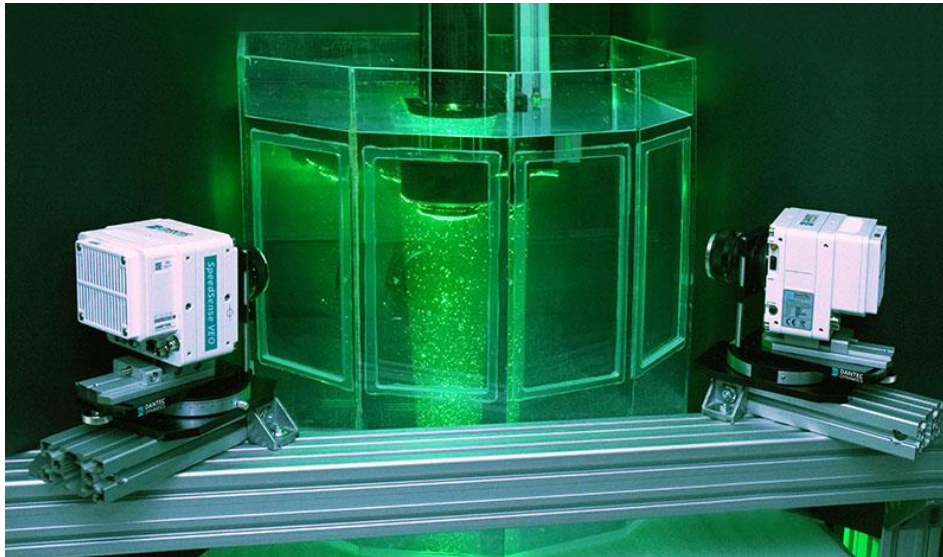
Johan le Nepvou de Carfort
PhD Student

Development of a digital twin for
mixing observation and optimization

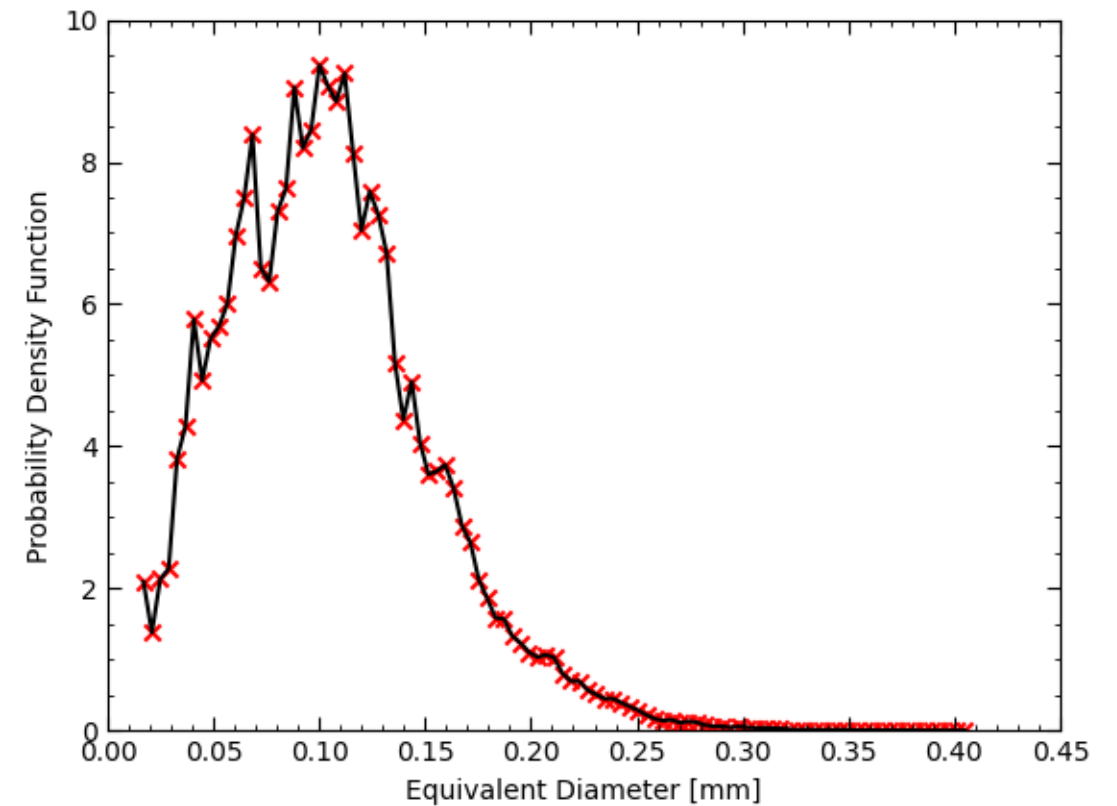
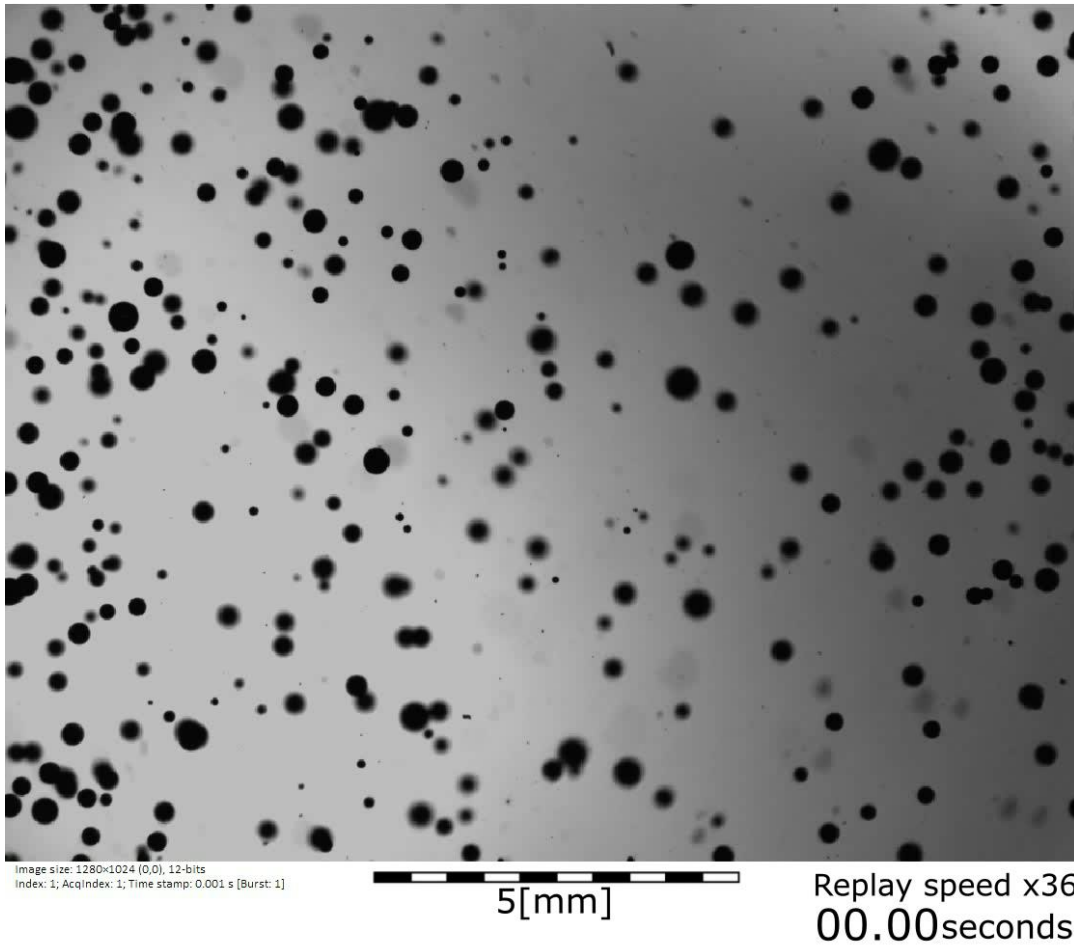


Particle Image Velocimetry (The next step) .

- Direct measurements of velocity field, turbulent dissipation rate and bubble velocity and size distribution.
- Allows for an accurate validation of the CFD models to predict large scale behavior, based on small scale measurements.



You see what you get:



Scaling up (and Down)

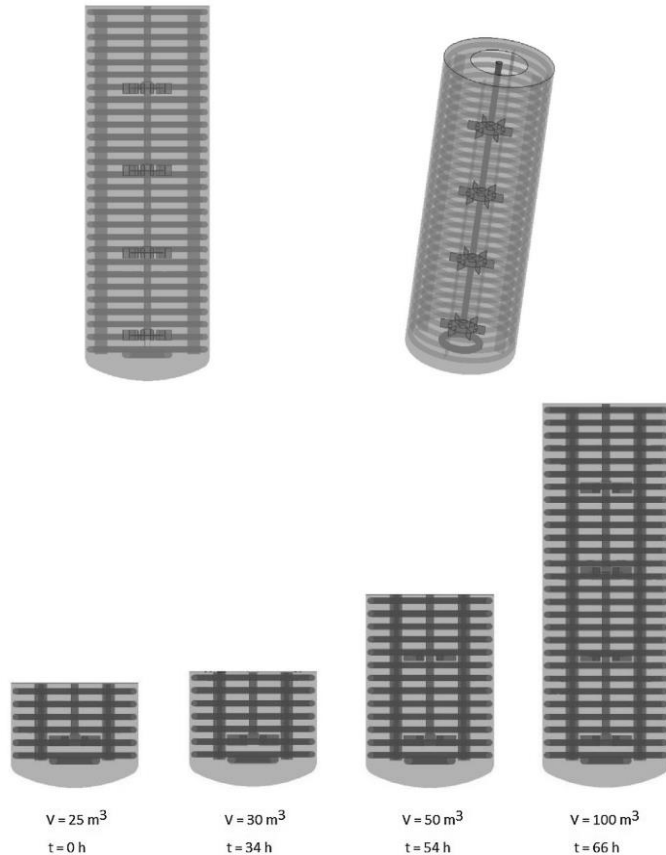


Fig. 7 – Illustration of the four volumes used for the simulation of different stages in the fed-batch operation of the bioreactor.

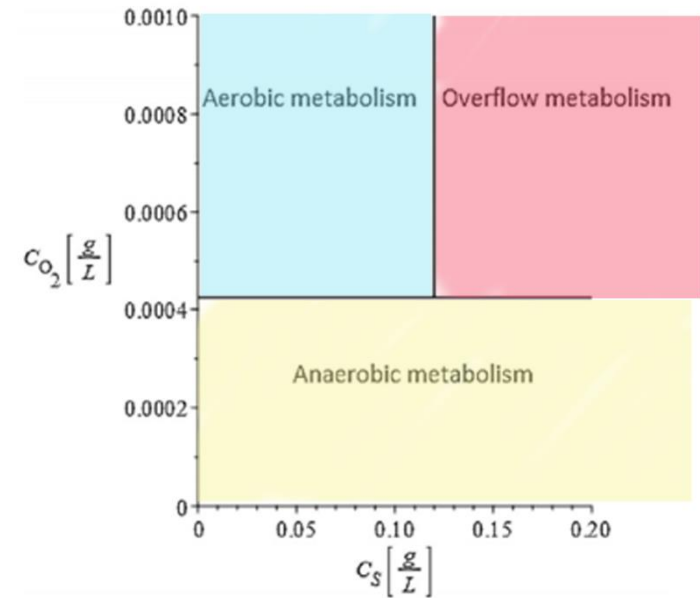


Fig. 3 – Boundaries between aerobic metabolism, overflow metabolism, and anaerobic metabolism defined in the kinetic model of *S. cerevisiae*.

Wright, M. R. *et al.* (2018) 'Investigation of the effect of uncertain growth kinetics on a CFD based model for the growth of *S. cerevisiae* in an industrial bioreactor', *Chemical Engineering Research and Design*. Institution of Chemical Engineers, 140, pp. 12–22. doi: 10.1016/j.cherd.2018.09.040

Scaling up (and Down)

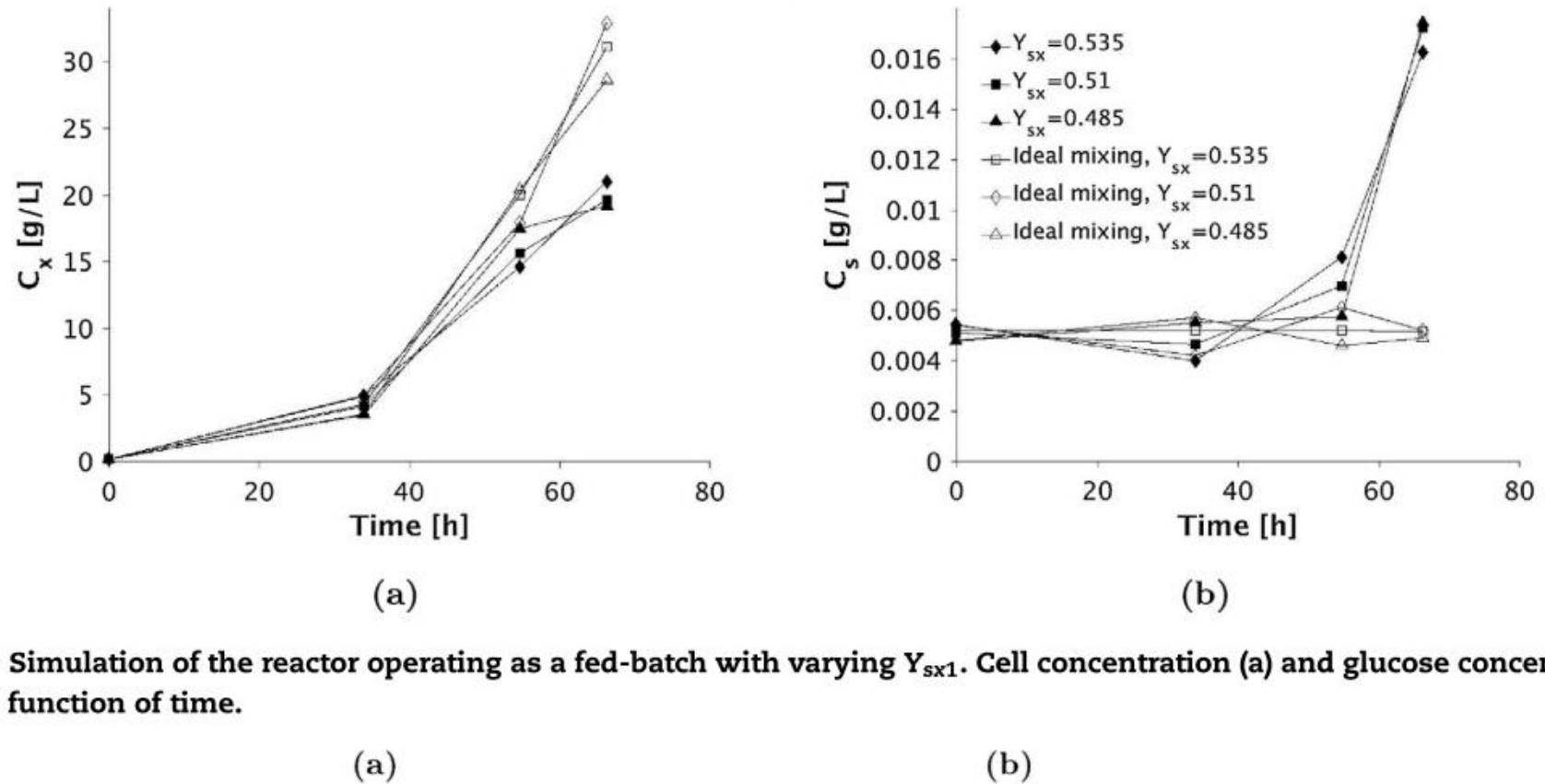
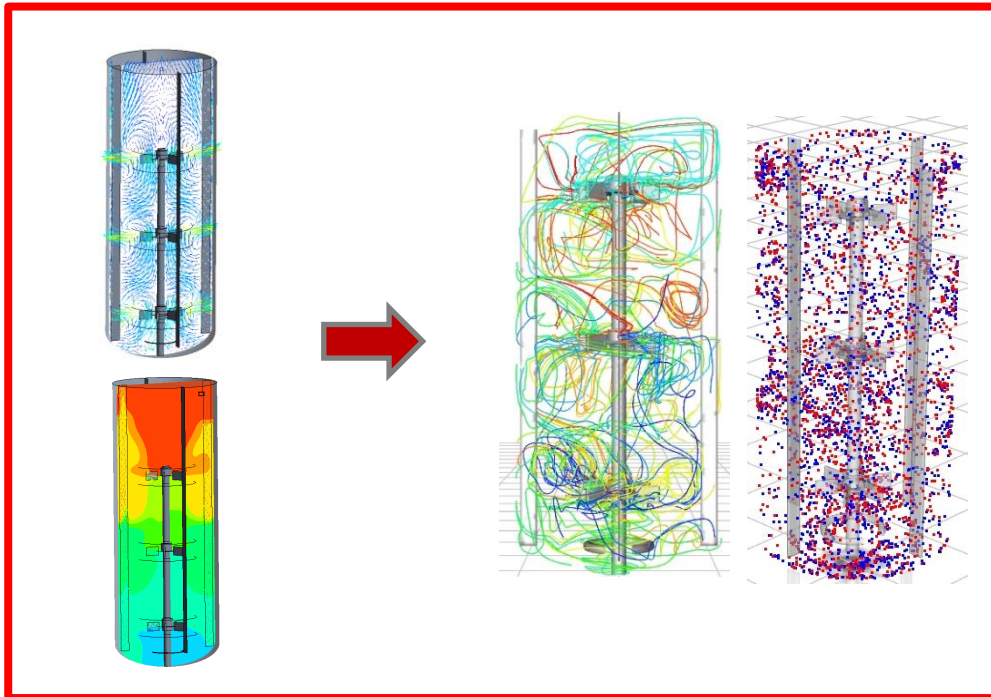


Fig. 9 – Simulation of the reactor operating as a fed-batch with varying Y_{sx1} . Cell concentration (a) and glucose concentration (b) as a function of time.

Wright, M. R. *et al.* (2018) 'Investigation of the effect of uncertain growth kinetics on a CFD based model for the growth of *S. cerevisiae* in an industrial bioreactor', *Chemical Engineering Research and Design*. Institution of Chemical Engineers, 140, pp. 12–22. doi: 10.1016/j.cherd.2018.09.040

CFD-Scaling of *Pseudomonas putida* Fermentation

- Developing a Euler-Lagrange model coupled with kinetic model for fed-batch fermentation of *P. putida* in a 200L pilot bioreactor
 - The simulations were performed at four different stages of the fermentation to account for the increase in the broth's height with continuous feeding and its effect on the mixing and gradient formation.
- Developing a Scale-Down simulator for bioprocesses through CFD life-line analysis



life lines/trajectories
Analysis



**Scale Down
Simulator**

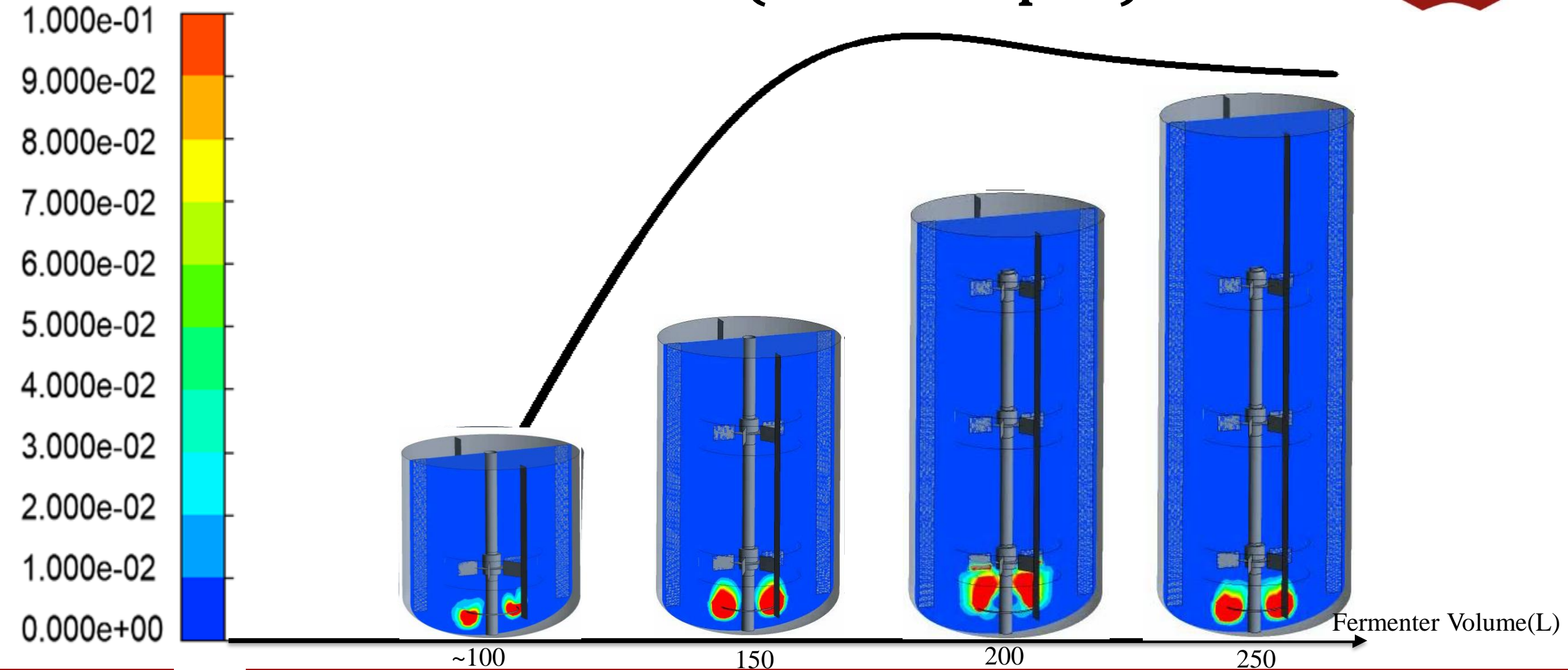


Mariyam Jamshidzadeh

Post Doctoral Fellow

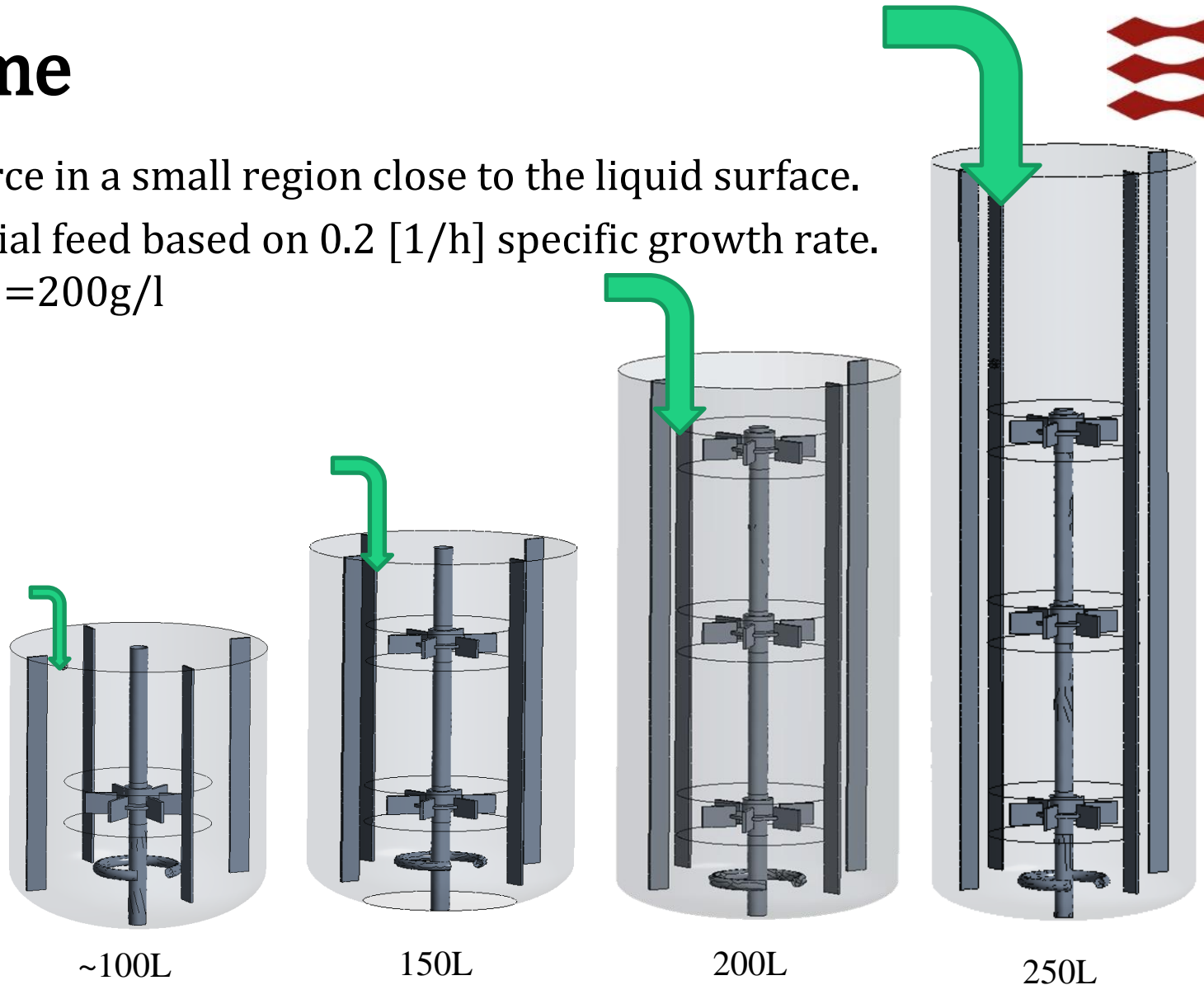
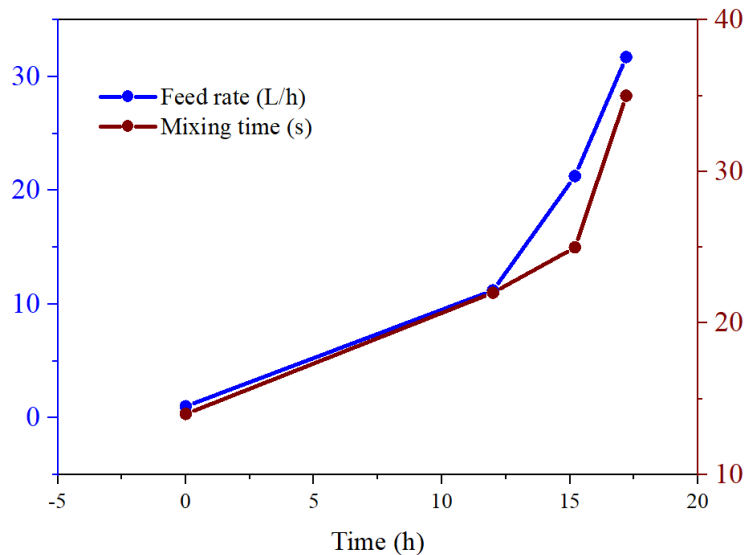
CFD kinetic Modelling for the Scale up
of *P. Putida* Fed Batch Fermentations

Air Volume Fraction (Gas Holdup-%)

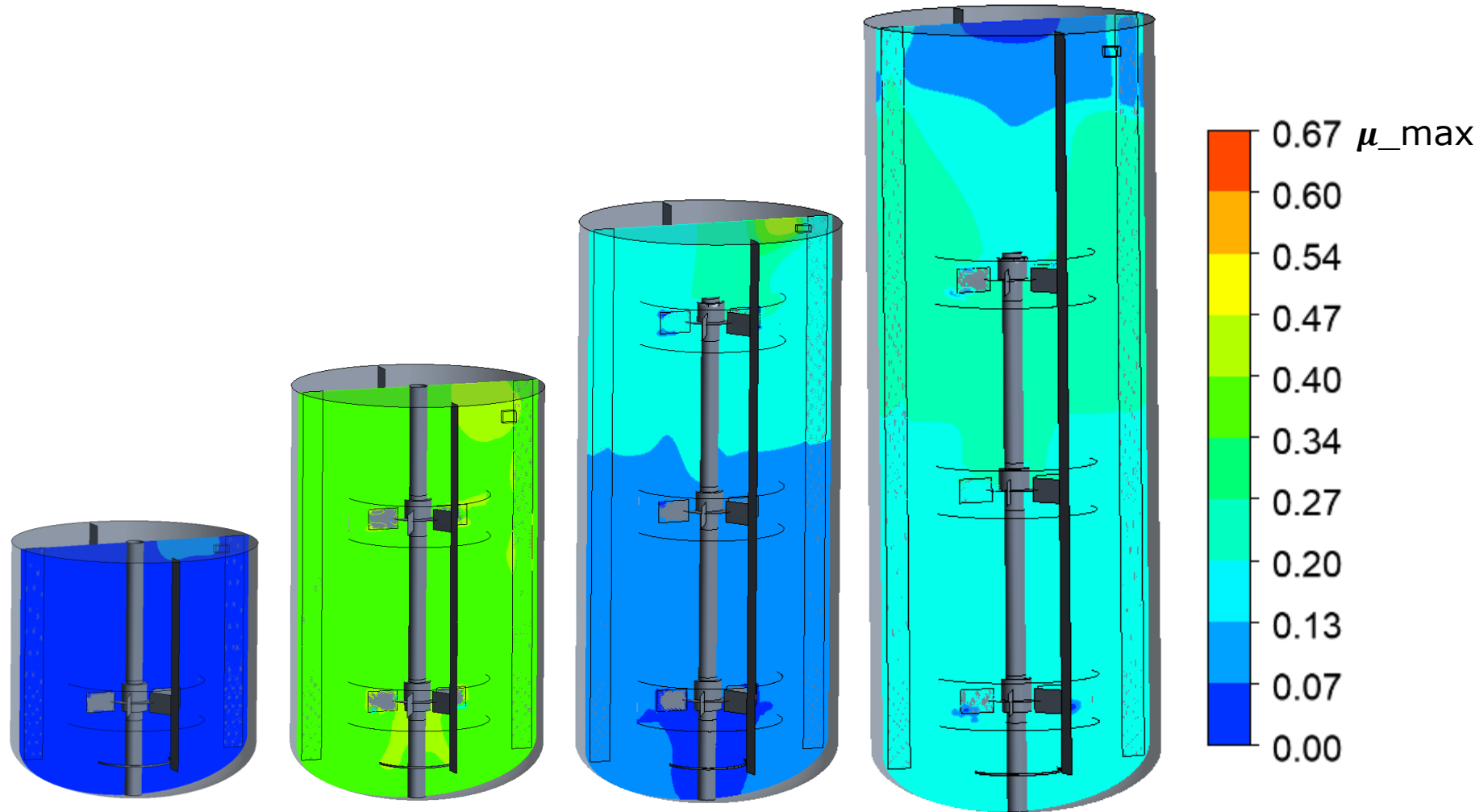
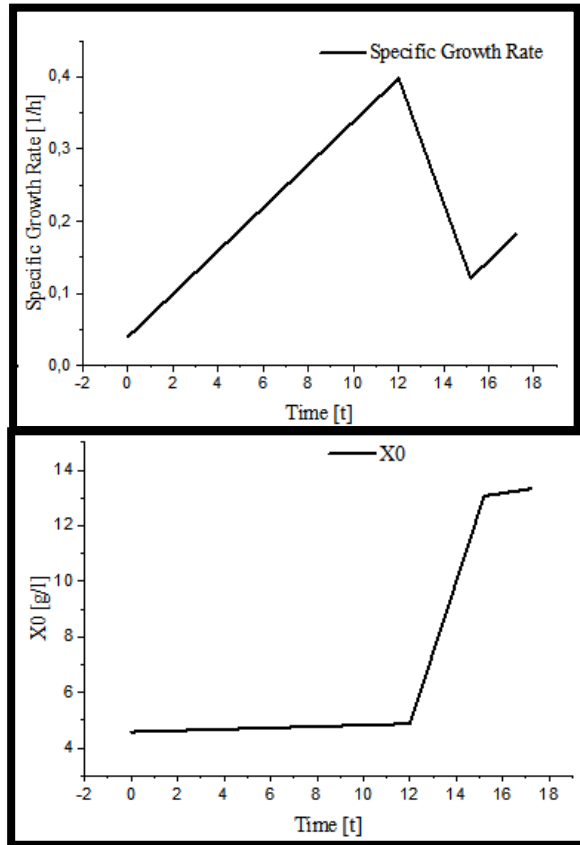


Feeding Scheme

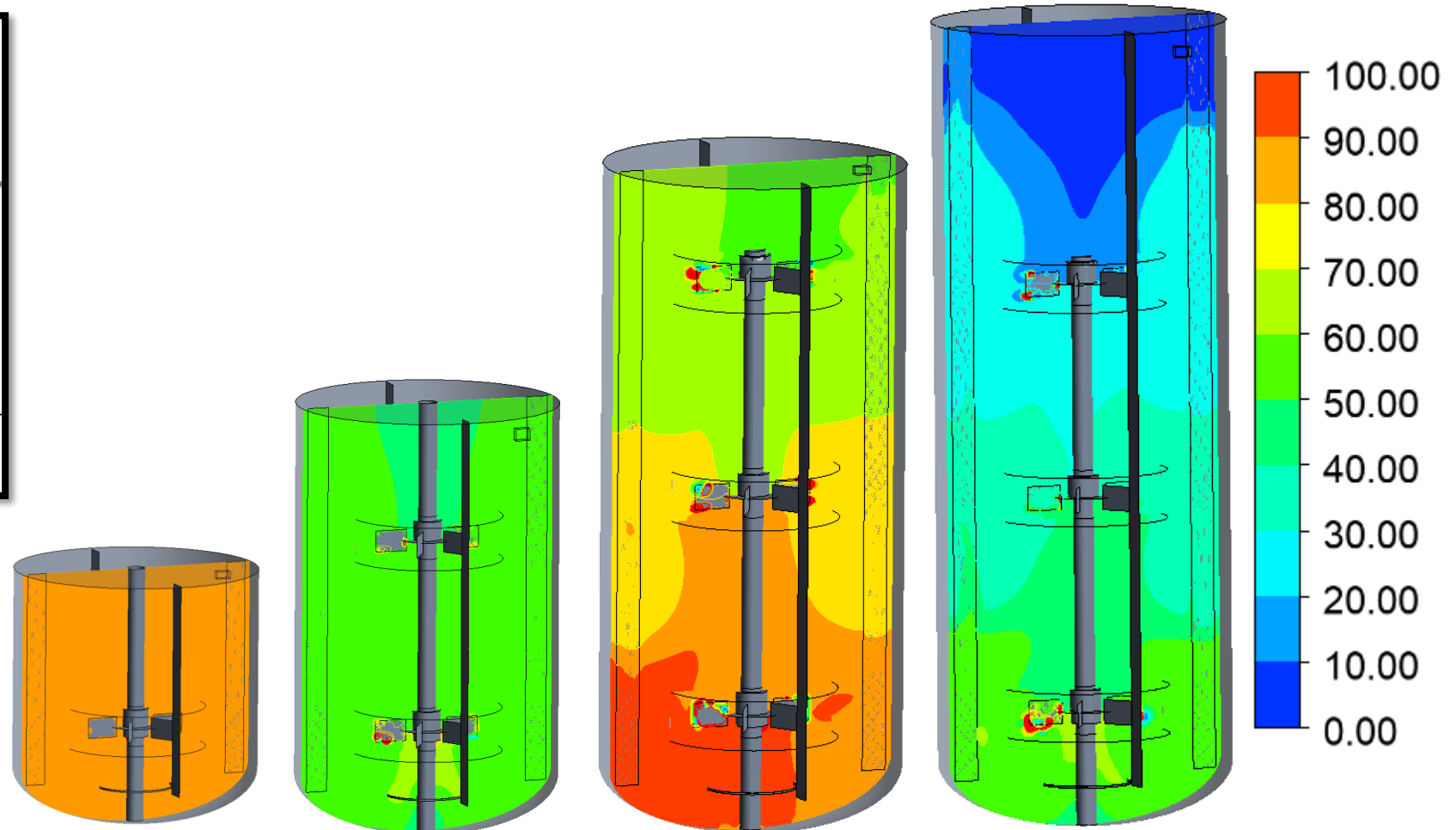
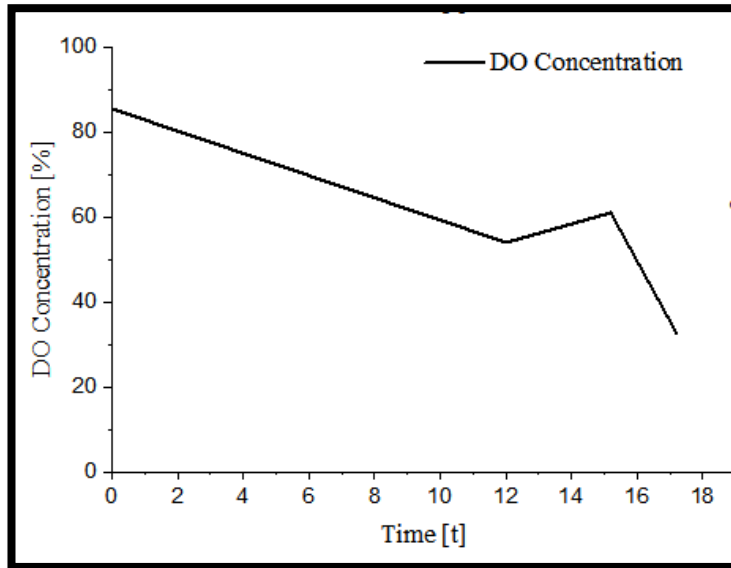
- Feed was added as a scalar source in a small region close to the liquid surface.
- Steady-state analysis: exponential feed based on 0.2 [1/h] specific growth rate.
Substrate concentration in feed = 200g/l



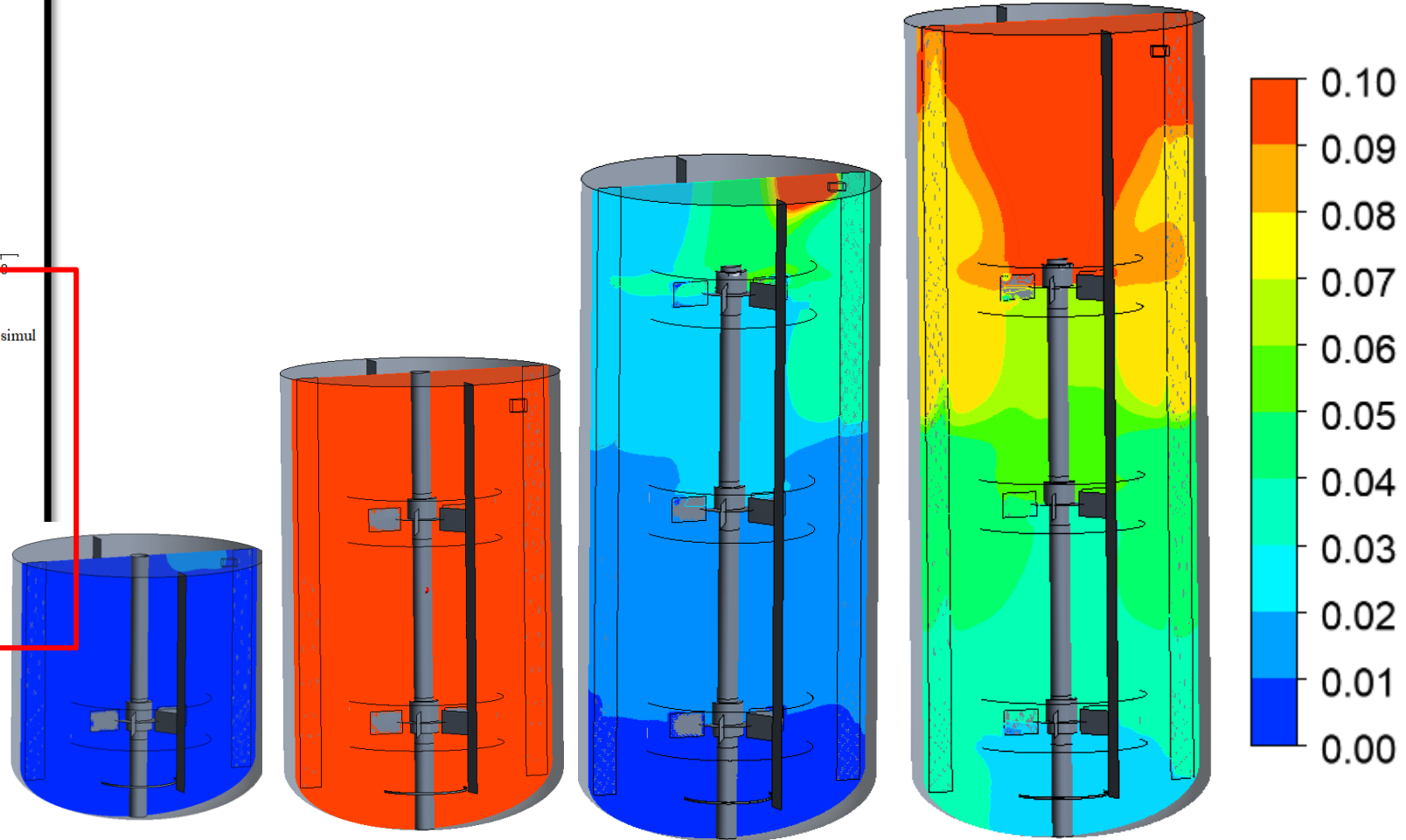
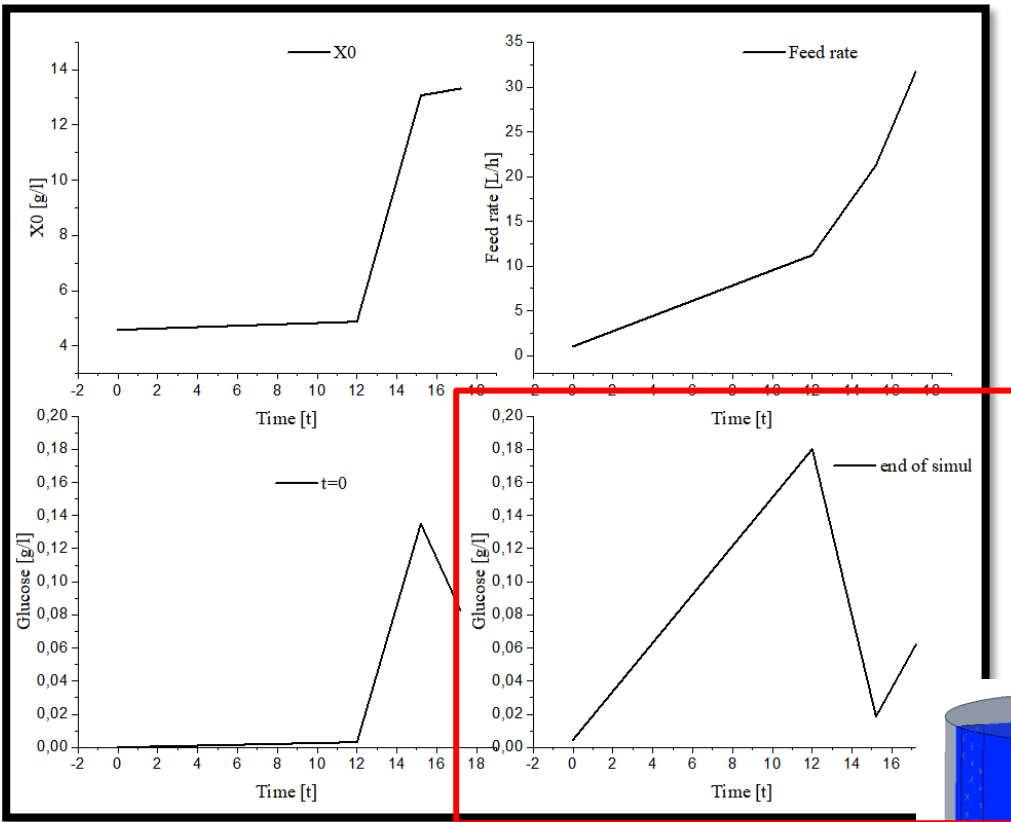
Specific Growth rate: μ [1/h]



Dissolved Oxygen (DO/Saturation DO-%)



Substrate Concentration [g/l]



Considering the past



Victor Puig I Labora

PhD Student

Department of Bioengineering

Making Cell-Factories first-class

citizens in Digital Twins of bioprocesses:

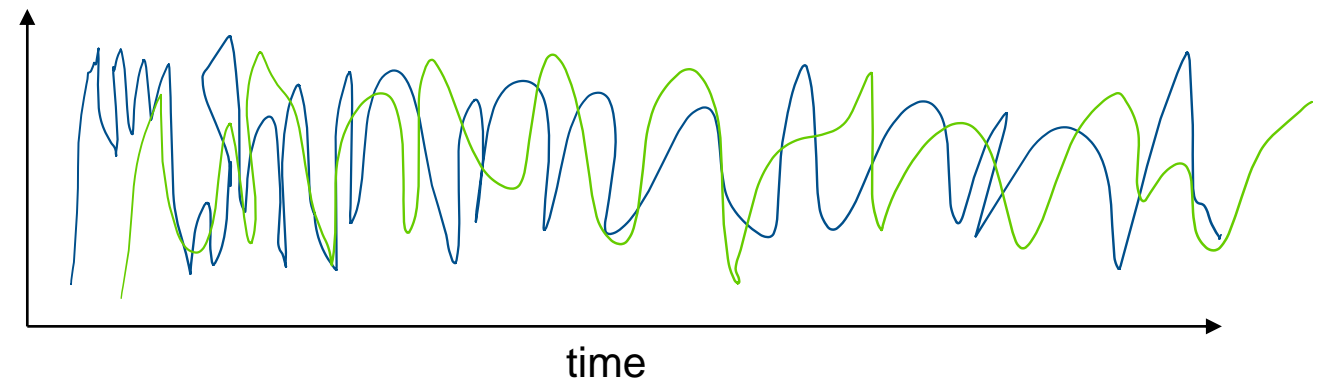
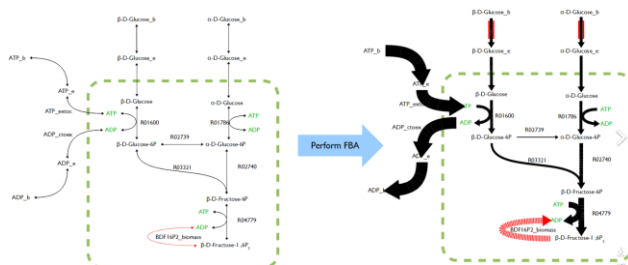
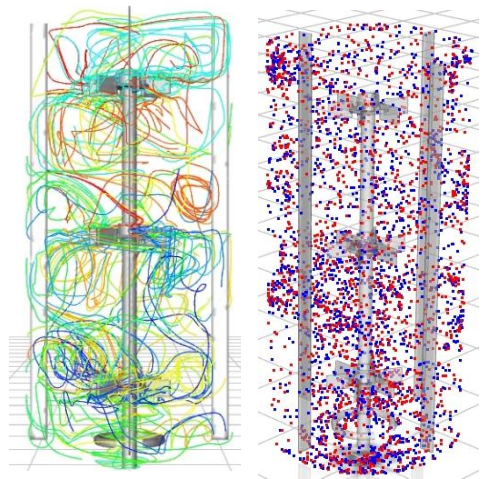
Integration of systems biology models

and CFD to support bioprocess scale

up

Condition
&
Performance

Flux balance analysis (FBA) is a mathematical method for simulating metabolism in genome-scale reconstructions of [metabolic networks](#). In comparison to traditional methods of modeling, FBA is less intensive in terms of the input data required for constructing the model. Simulations performed using FBA are computationally inexpensive and can calculate steady-state metabolic fluxes for large models (over 2000 reactions) in a few seconds on modern personal computers. The related method of [metabolic pathway analysis](#) seeks to find and list all possible pathways between metabolites.



Compartment models

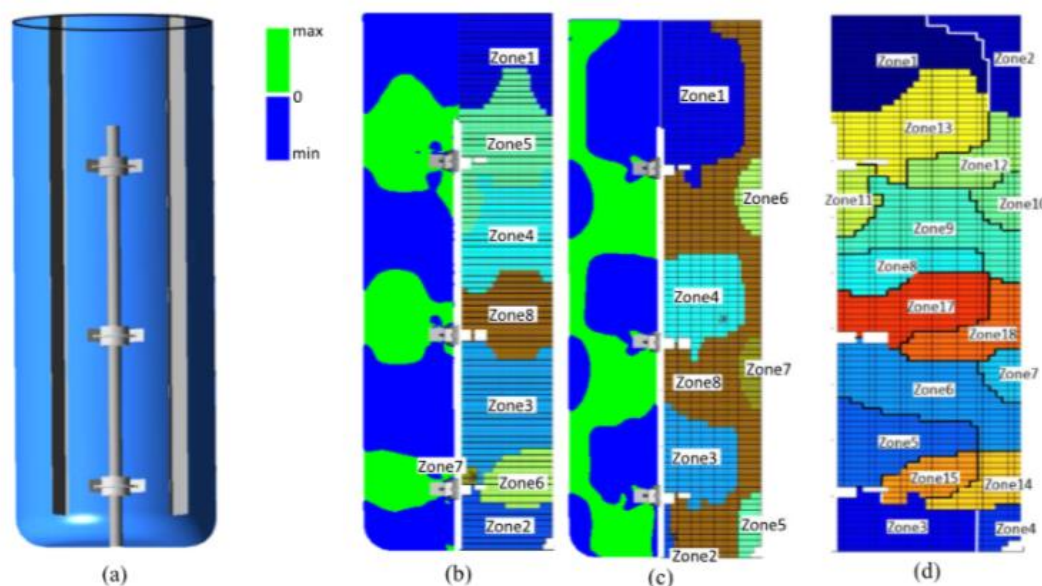


Fig. 6. Compartmentalization of the 700L bioreactor at 300 rpm. (a) geometrical configuration; (b) CFD simulation of radial velocity (left) vs. zoning map based on radial velocity (right); (c) CFD simulation of axial velocity (left) vs. zoning map based on axial velocity (right); (d) final compartment-map based on combined radial and axial velocities (the x-dimension is enlarged for better visualization).

Tajsoleiman, T., Spann, R., Bach, C., Gernaey, K. V., Huusom, J. K., & Krühne, U. (2019). A CFD based automatic method for compartment model development. *Computers and Chemical Engineering*, 123, 236–245.

<https://doi.org/10.1016/j.compchemeng.2018.12.015>

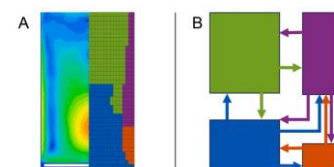


Fig. 8. (A) The showing (A) CFD simulation contour plot result showing the velocity profile of a bubble column bioreactor and its accompanying compartment map on the right side developed with an automatic compartment map development method based on the axial and radial velocity fields (Tajsoleiman et al., 2019). (B) Representation of the resulting network of zones with connections that can be used in modelling work for accounting for the fluid dynamics of the bubble column.

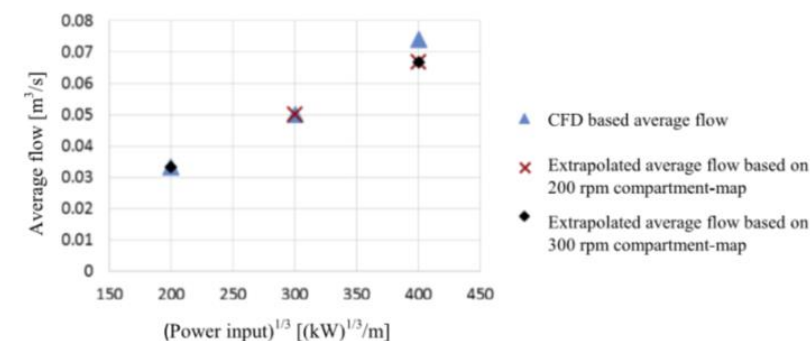


Fig. 9. Extrapolated average exchange flow compared to the extracted flows from the CFD simulations.

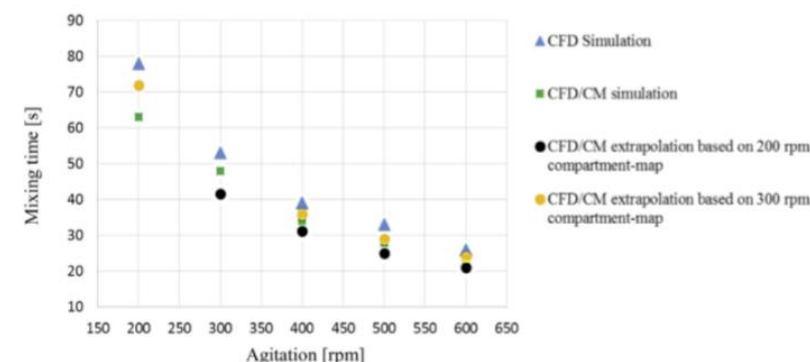


Fig. 10. Estimation of mixing time for different agitation speeds by the extrapolated flow.

Understanding gradients in industrial bioreactors

Gisela Nadal-Rey^a, Dale D. McClure^b, John M. Kavanagh^b, Sjef Cornelissen^c, David F. Fletcher^b, Krist V. Gernaey^{a,*}

^a Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, Building 228A, 2800 Kgs. Lyngby, Denmark

^b The University of Sydney, School of Chemical and Biomolecular Engineering, Building J01, Camperdown 2006, NSW, Australia

^c Novozymes A/S, Fermentation Pilot Plant, Krogshøjvej 36, 2880 Bagsvaerd, Denmark

Fermentation needs purification

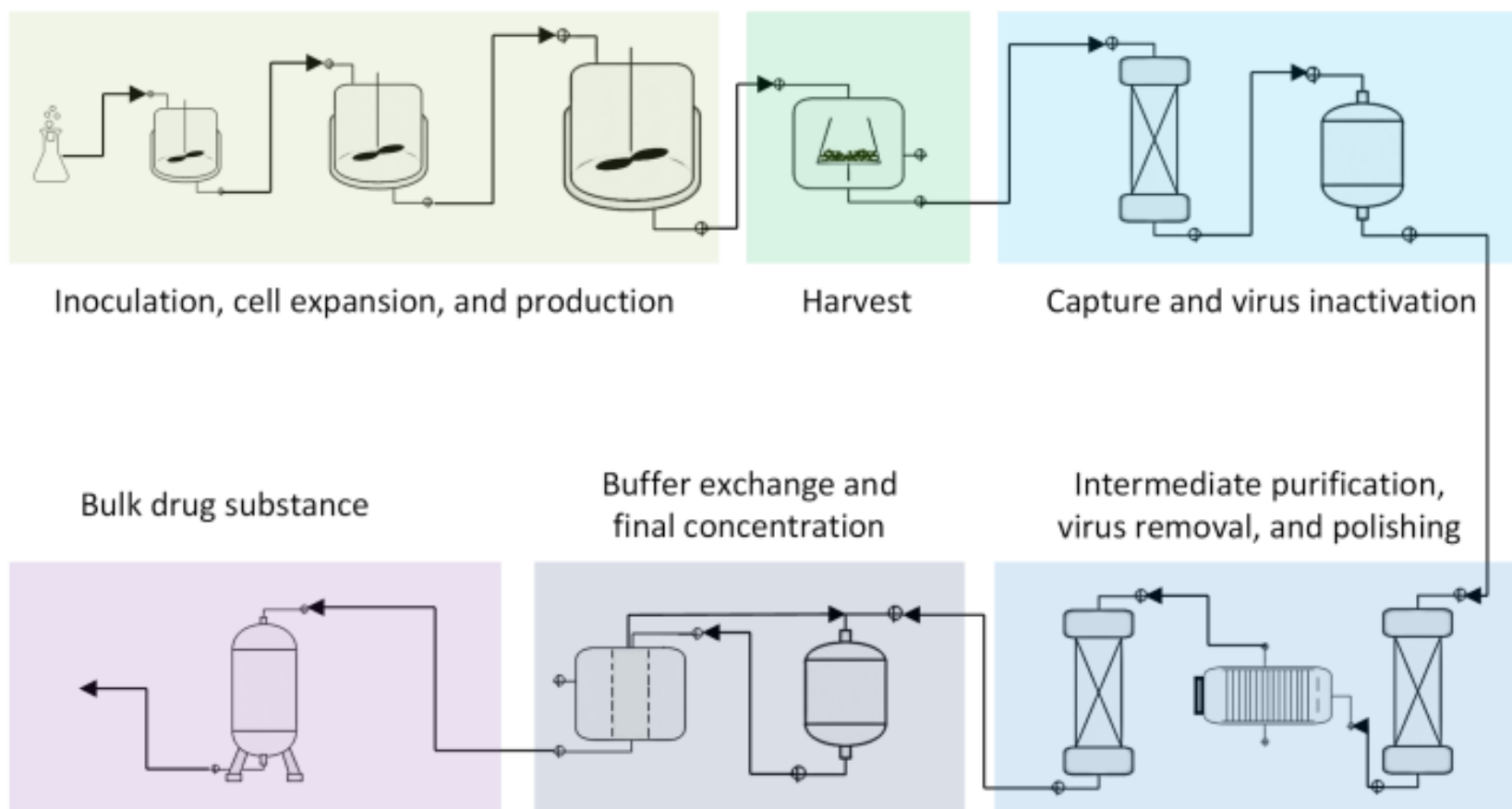
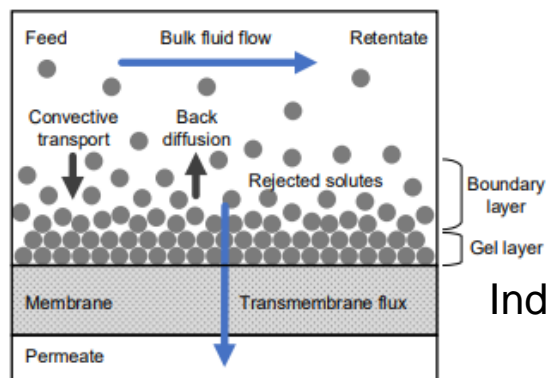


Figure 1.2: Exemplary mAb manufacturing platform process.

Other areas in bioprocesses: filtration



Industrial PhD student
Johann Kaiser

Fig. 2. Schematic cross-sectional view of a membrane cassette. The different cassette compartments and solute layers contributing to the resistance to the transmembrane flux are shown.

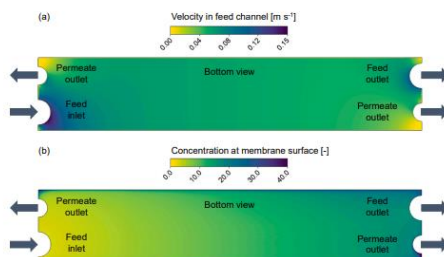


Fig. 12. Velocity and concentration distribution in a membrane cassette. (a) Fluid velocity in the feed channel, (b) concentration of a retained component at the membrane surface. For illustration purposes the mesh has been refined and the legend for the concentration plot has been adjusted such that the maximum shown value is equal to a concentration of 40. The maximum calculated value was 72.3 near the permeate outlet at the retentate side. The concentration range corresponds to measured viscosity values of approx. 1.0–1.8 mPa s. Feed flux = 257 L m⁻² h⁻¹, feed concentration = 3, retentate pressure = 0.3 bar.

Defining the optimal operating conditions and configuration of a single-pass tangential flow filtration (SPTFF) system via CFD modelling

Johann Kaiser^{a,b}, Janus Krarup^b, Ernst Broberg Hansen^b, Manuel Pinelo^a, Ulrich Krühne^{a,*}

^a Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søtofts Plads, 2800 Kgs. Lyngby, Denmark

^b CMC API Development, Novo Nordisk A/S, Smørumsevej 17, 2880 Bagsvaerd, Denmark

Industrial PhD student
Henrik Sander Marke

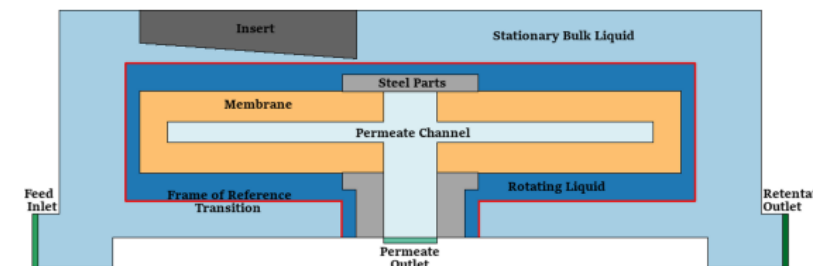


Fig. 2. Conceptual sketch of the simulated geometry. Outlining the conceptual parts in the simulated geometry. In green colors: Inlet and outlets. In blue colors: Free flowing liquid domains. In yellow: The membrane. In grey: Steel parts. The red line denotes the transition from a stationary frame of reference to a rotating frame of reference. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

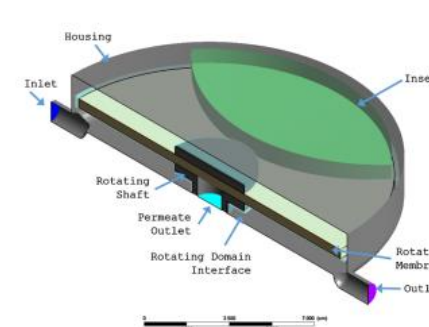


Fig. 1. Scaled Drawing of the simulated geometry. Half Plane of the simulated geometry. Features: Dark Blue: Inlet. Purple: Outlet. Light Grey: Housing, stationary walls. Dark Grey: Shaft, rotating walls. Light Blue: transition domain interface. Orange: Rotating membrane. Teal: Permeate outlet. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

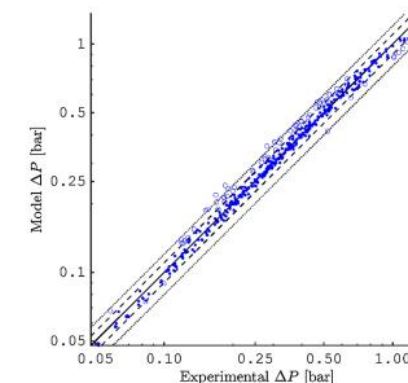


Fig. 12. Log Log Parity plot, of Experimental ΔP vs model ΔP . Experimental ΔP and corresponding Model ΔP . Distance from solid centerline reflects deviation between model and measured data. Dotted line: 20% deviation between data and model value. Dashed line: 10% deviation between data and model value.

Investigation of the velocity factor in a rotational dynamic microfiltration system

Henrik S. Marke^{a,b}, Martin P. Breil^b, Ernst Broberg Hansen^b, Manuel Pinelo^a, Ulrich Krühne^{a,*}

^a Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søtofts Plads 229, 2800 Kgs. Lyngby, Denmark

^b Novo Nordisk A/S, Bagsvaerd, Denmark

Conclusion and opportunities for Scale down?

- Compartment Models instead CFD
- Compartment Reactors in order to represent the reality. How far can we go?
 - Do we build tailor suited single reactors?
 - Use available commercial systems in networks? (AMBR, M2lab, etc?)
 - What do we get out here?
 - How is the organism dealing with the changes?

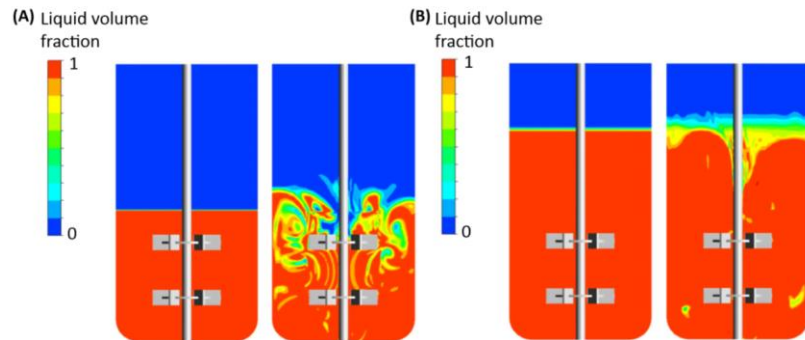
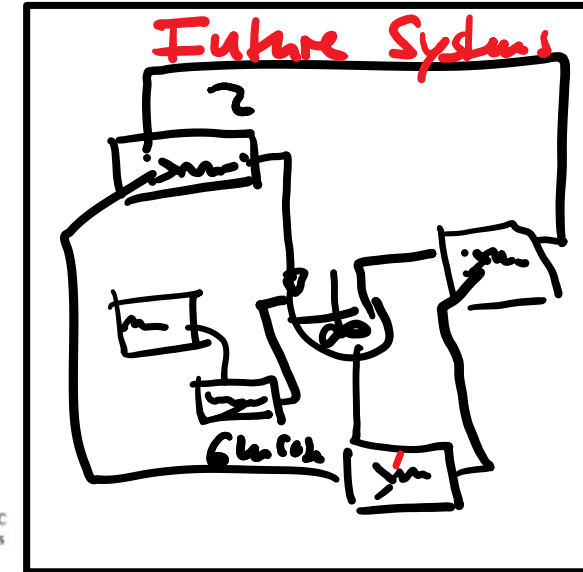
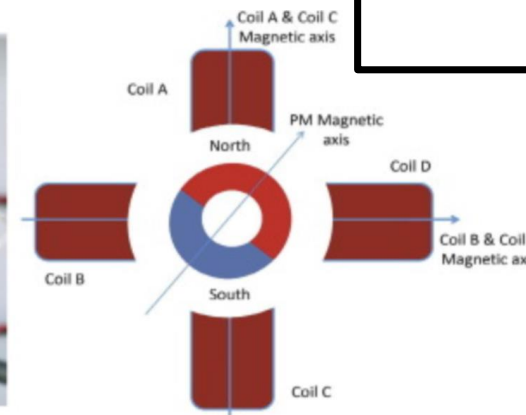
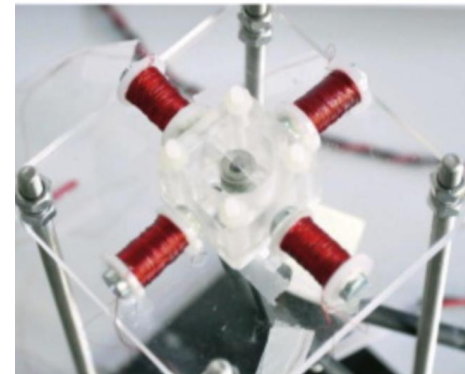


Figure 2. Computational Fluid Dynamics Simulation of Vortex in Water. (A) With 150 ml, and (B) 250 ml working volume without agitation (left-hand side) and 800 rpm agitation speed (right-hand side) with no aeration.



Trends in Biotechnology

Tajsoleiman, T., Mears, L., Krühne, U., Gernaey, K. V., & Cornelissen, S. (2019). An Industrial Perspective on Scale-Down Challenges Using Miniaturized Bioreactors. *Trends in Biotechnology*, xx, 1–10. <https://doi.org/10.1016/j.tibtech.2019.01.002>

Acknowledgements

- **PhD students**

- Johann Kaiser
- Johan le Nepvou de Carfort
- Henrik Sander Marke
- Tannaz Tajssoleiman
- Christian Bach

- **MSc Students**

- Mathias Wright

- **Colleagues**

- Prof. John Woodley
- Prof. Krist Gernaey
- Ines Pereira Roshinha Grundtvig

- **Post Doc**

- Maryiam Jamshidzadeh

- **International**

- Prof. Polona Žnidaršič Plazl
- Prof. Igor Plazl
- Prof. Torsten Mayr
- Prof. Gerardo Perozziello
- Prof. Rainer Krull
- Prof. Nicolas Szita
- Dr. Adama Sesay

