TECHNISCHE UNIVERSITÄT WIEN

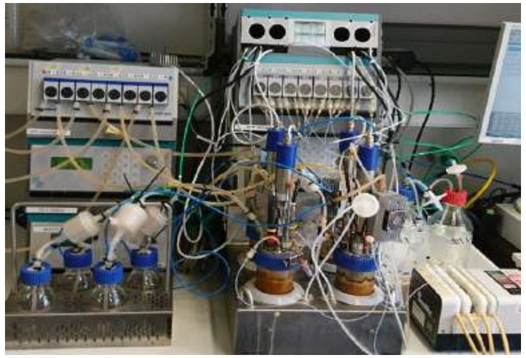
iFermenter INTELLIGENT CELLS

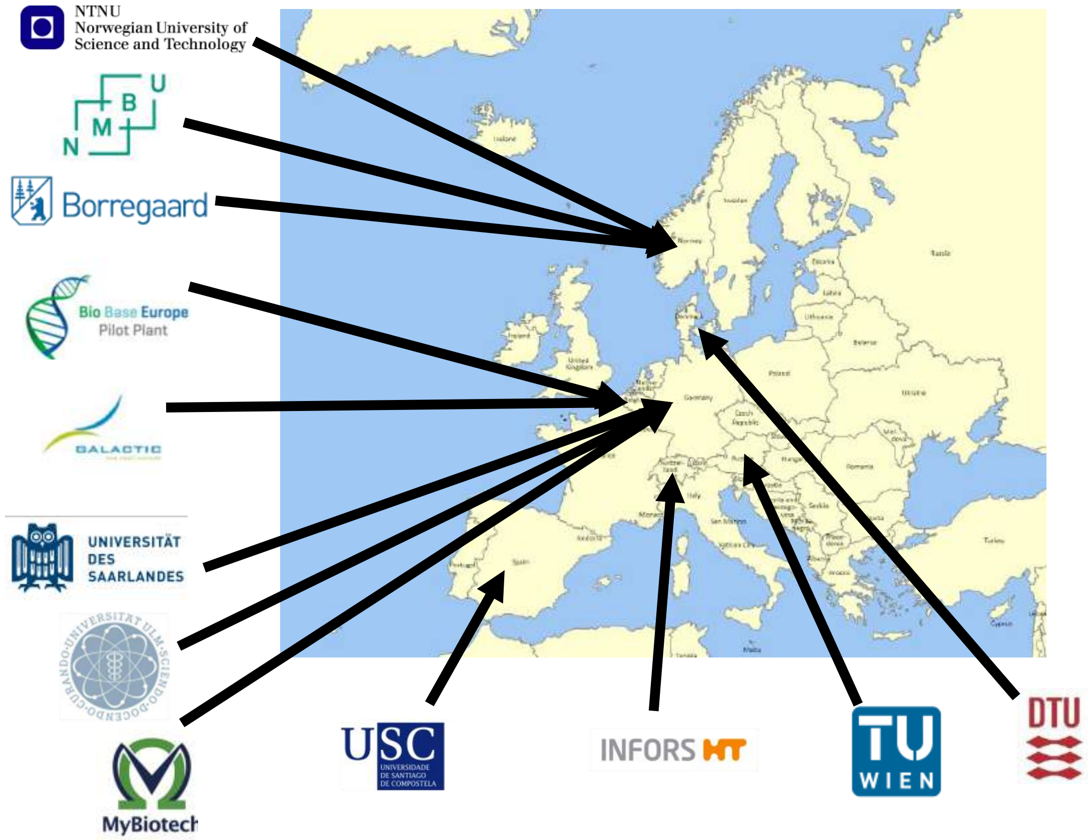
Modellgestützte Bioprozessentwicklung zur effizienten Nutzung von Sulfitablauge

Peter Sinner, Julian Kager, Sven Daume, Christoph Herwig

iFermenter project

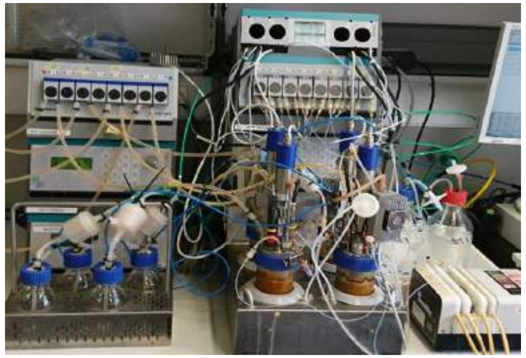
- New BBI (<u>B</u>io-<u>B</u>ased <u>I</u>ndustries) project
- Converting residual sugar streams (SSL, BALI) from pulp industry to high value products

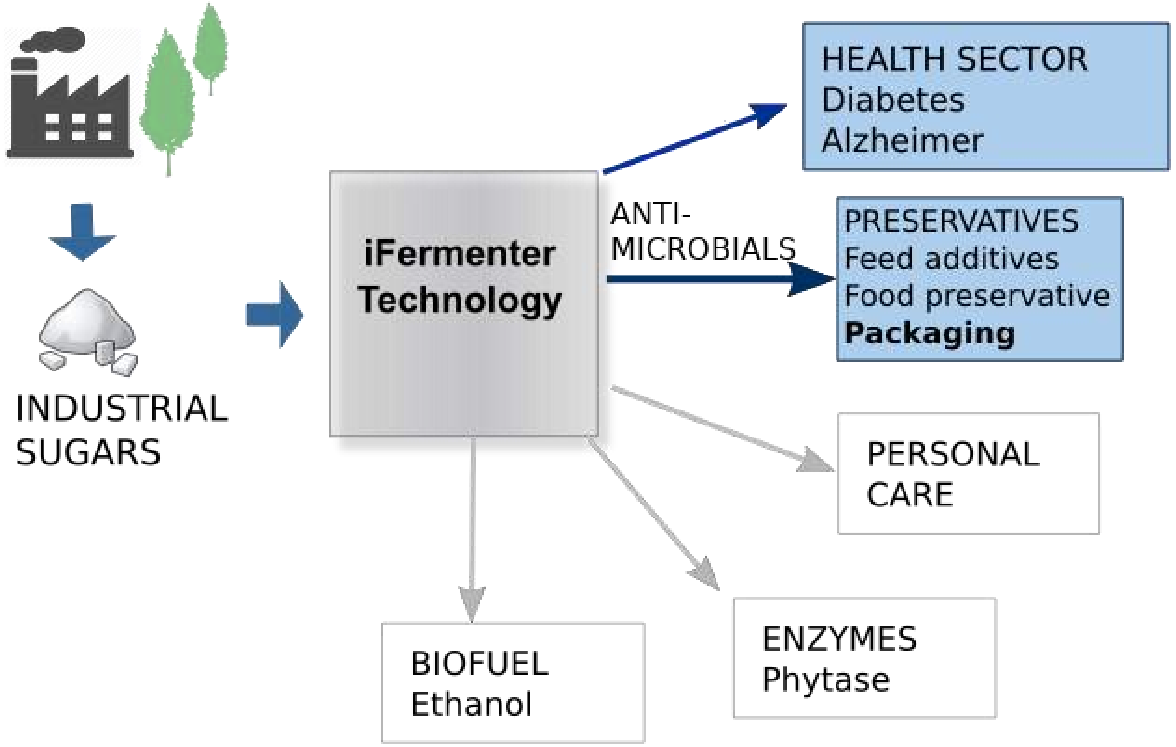




iFermenter project

- New BBI (Bio-Based Industries) project
- Converting residual sugar streams (SSL, BALI) from pulp industry to high-value products



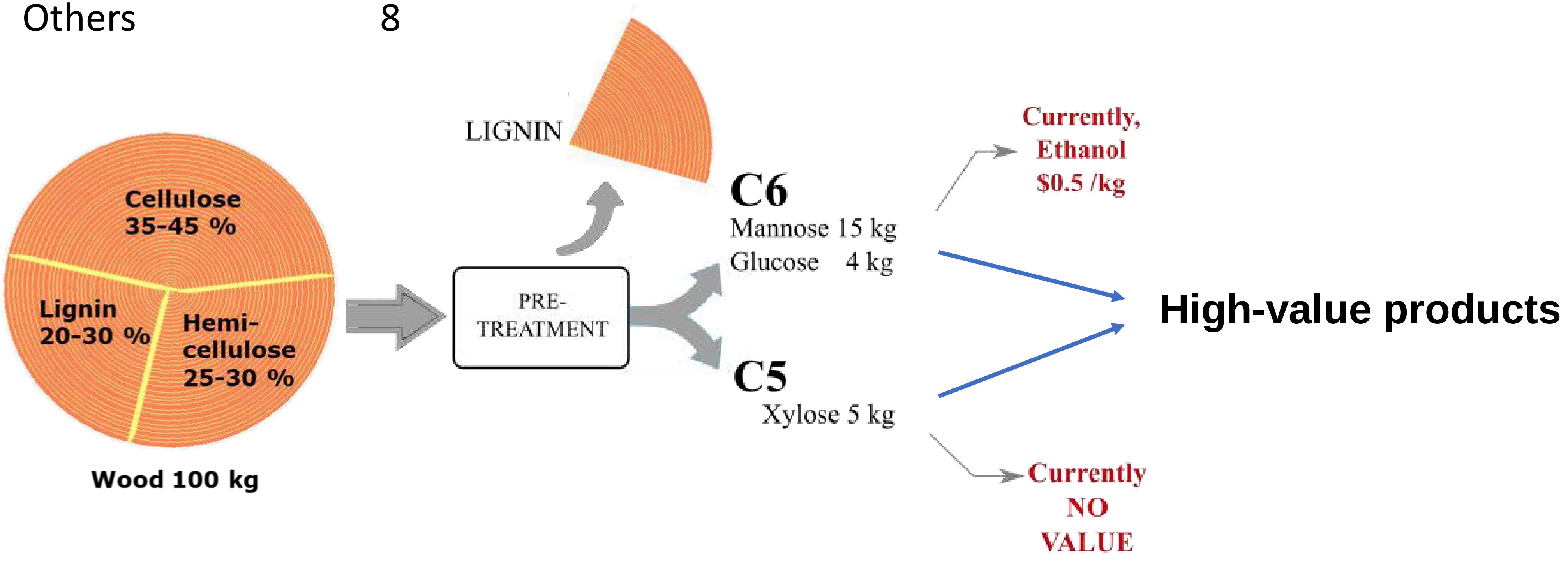


SSL composition

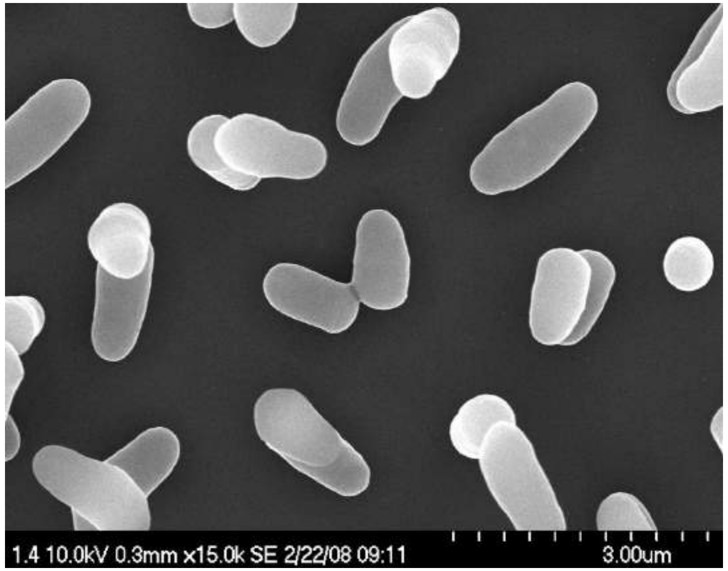
Sugar	%
Mannose	30
Xylose	10
Glucose	8
Others	8

BALI composition

Sugar	%
Glucose	85.0
Fructose	2.0



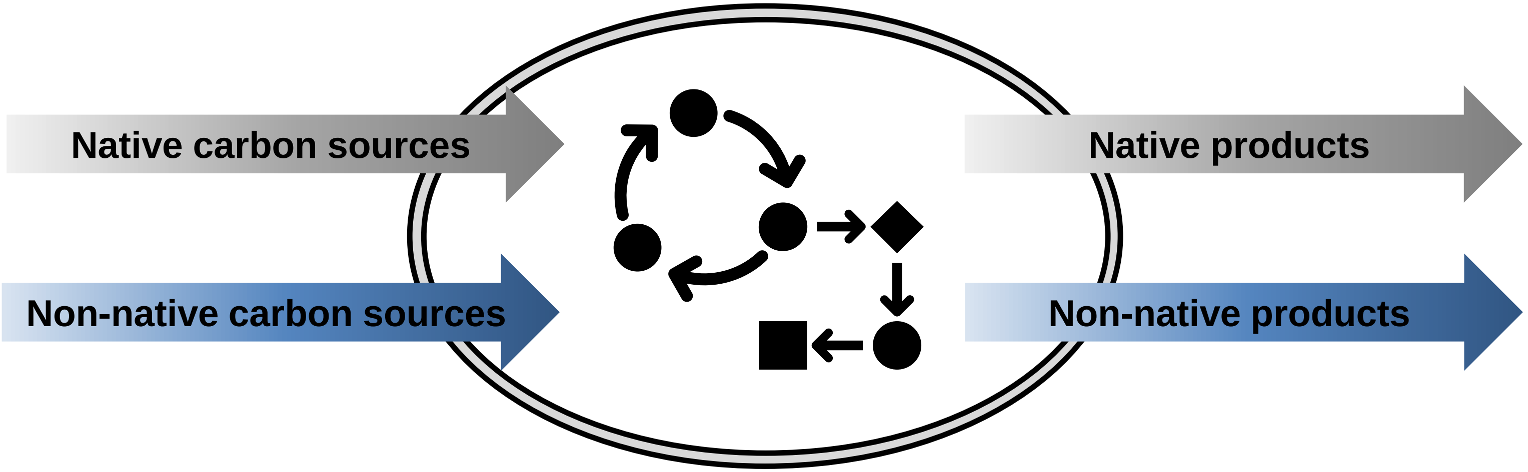
Corynebacterium glutamicum



Gerd Seibold (DTU)

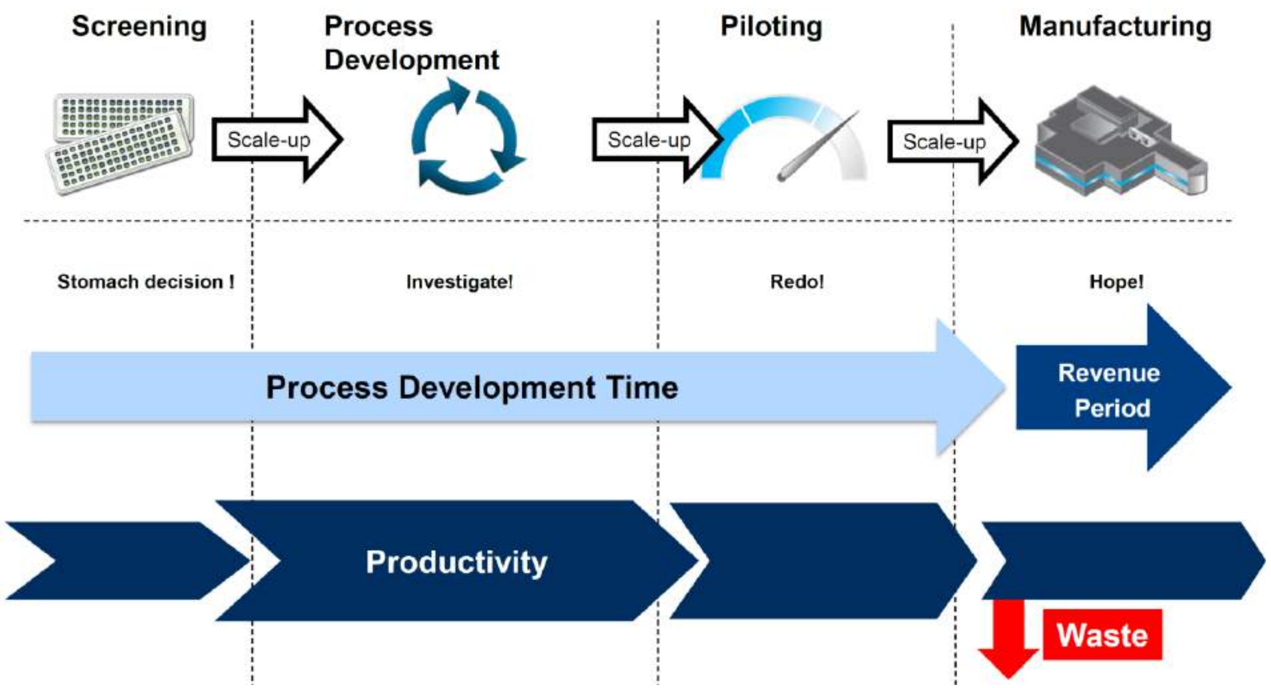
- Gram-positive, facultative anaerobic, GRAS
- Extensively metabolic engineered

Corynebacterium glutamicum cell

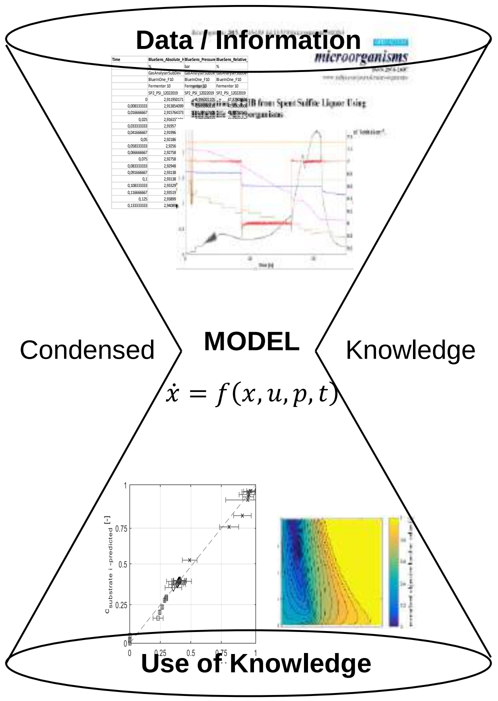


New strategies in bioprocess development

- Status quo of bioprocess development: trial-and-error
- **Goal:** design of **productive** and **sustainable** processes based on quantitative process understanding

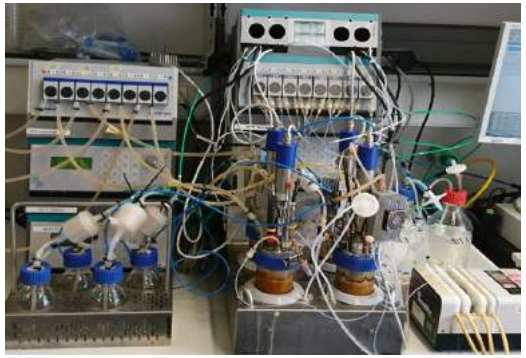


➡ Use of dynamic models during the entire process life cycle



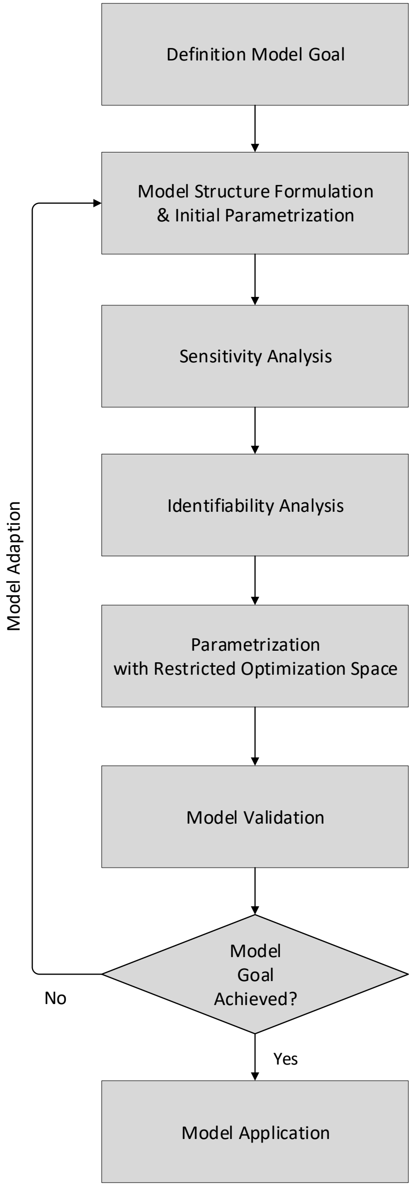
Bioprocess case study

- Utilizilation of SSL by wildtype *Corynebacterium glutamicum*
- Target: **continuous processing**
  (maximum biomass space time yield & minimal residual carbon substrate loss)



- **Modelling of experimental data**
- **Model-based process design**
- **Linking process and strain engineering**

Modelling along Good Modelling Practice



NRMSE < 15 % for target states (biomass, sugar substrates)

Model applicable for design of continous process

ODE mass balances $\dot{x} = f(x, u, p, t)$

Parameter perturbation

Importance ranking

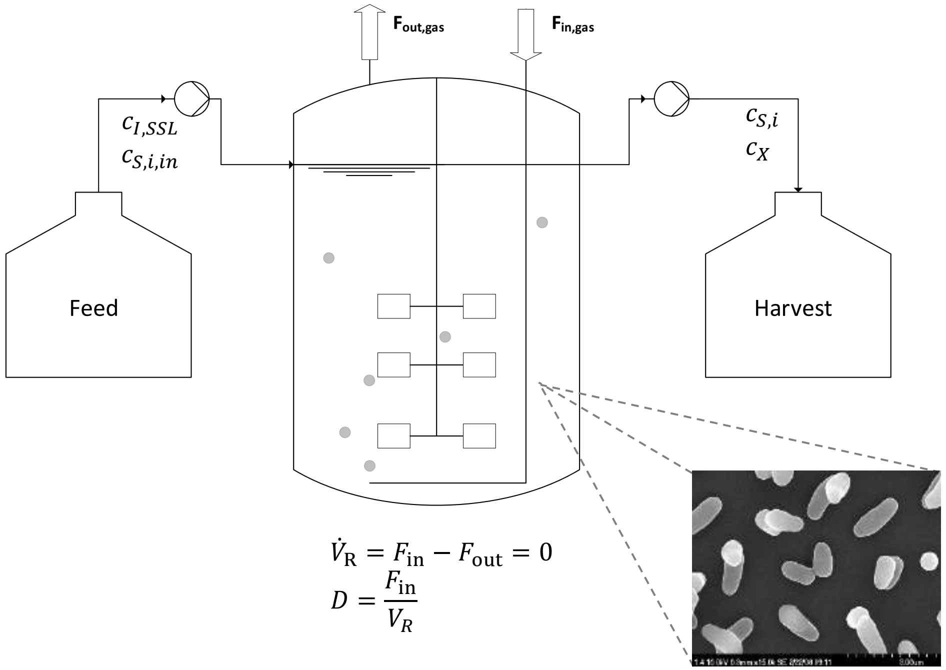
Practical identifiability

Genetic algorithm & constrained Nelder Mead

Multi-objective optimization

for process design

Modelling goal must be consistent with process goal!



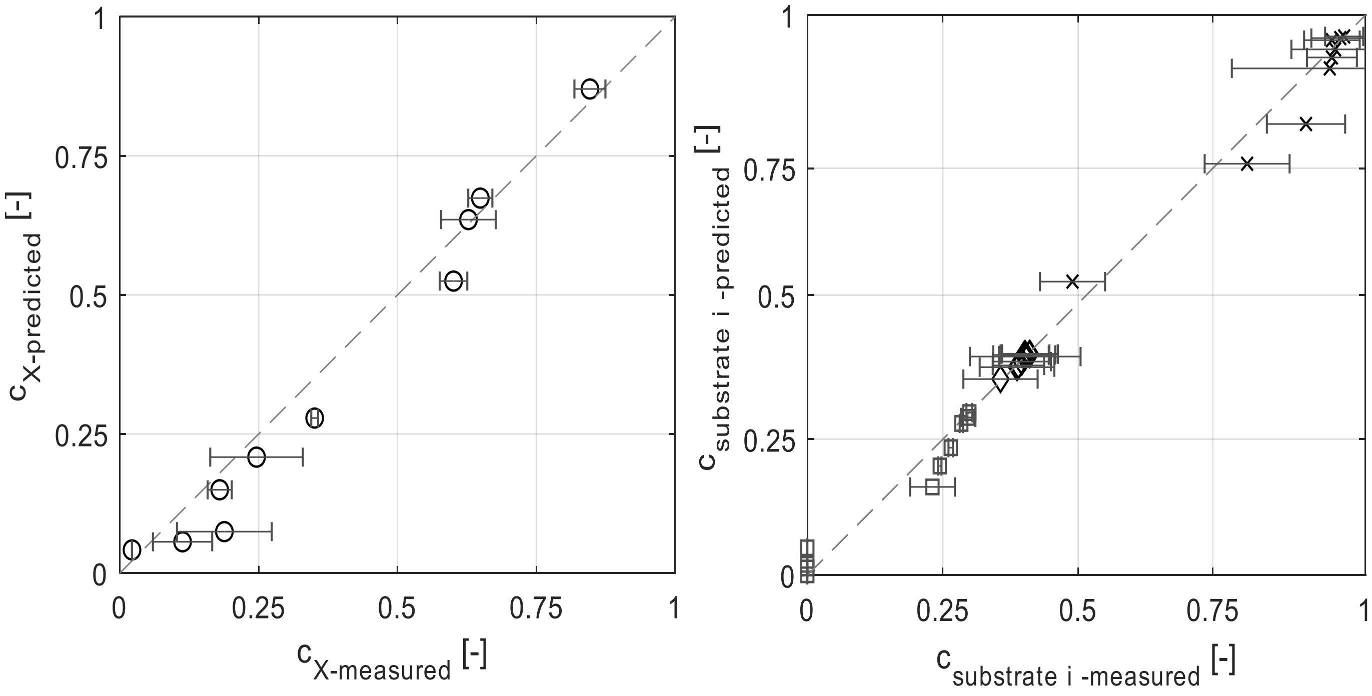
$\dot{V}_R = F_{in} - F_{out} = 0$

$D = \frac{F_{in}}{V_R}$

Gerd Seibold (DTU)

Modelling

- Parametrization & model validation



NRMSE on states ~ 10 %

State	NRMSE in %
Biomass concentration	6.73
Glucose concentration	11.0
Mannose concentration	9.11
Xylose concentration	8.40

Sinner et al., Model-based Analysis and Optimisation of a Continuous Corynebacterium glutamicum Bioprocess Utilizing Lignocellulosic Waste, IFAC PapersOnLine 52-26 (2019)
https://doi.org/10.1016/j.ifacol.2019.12.255

Model-based process design

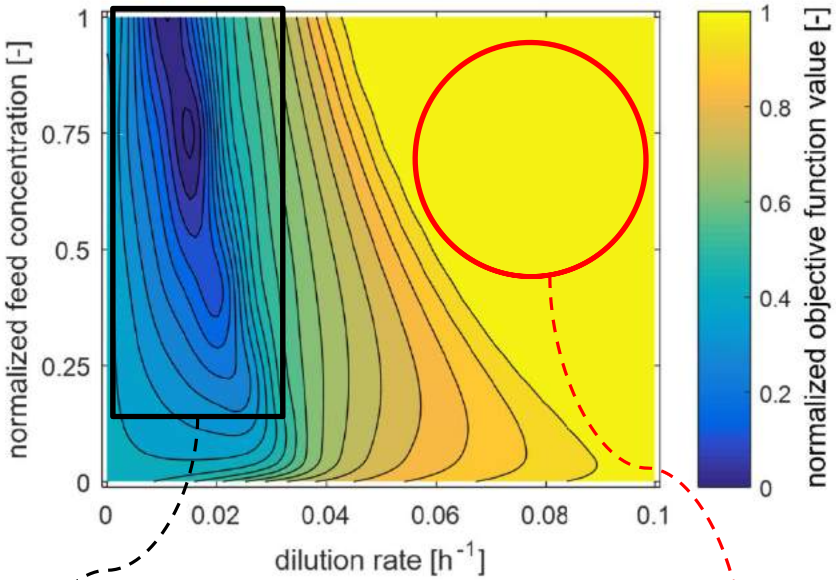
- Optimal operating conditions (dilution rate, concentration of waste in feed) for continuous bioprocess

$$\min_{D, c_{I,SSL}} J_1 = -c_{X,\text{steady state}} D$$

J_1: max. space time yield biomass production [g L^{-1} h^{-1}]

$$\min_{D, c_{I,SSL}} J_2 = \frac{\sum_{i=1}^{3} c_{S,i,\text{steady state}}}{c_{S,1,\text{in}} + c_{S,2,\text{in}} + c_{S,3,\text{in}}}$$

J_2: min. sugar loss to effluent relative to input concentration [-]



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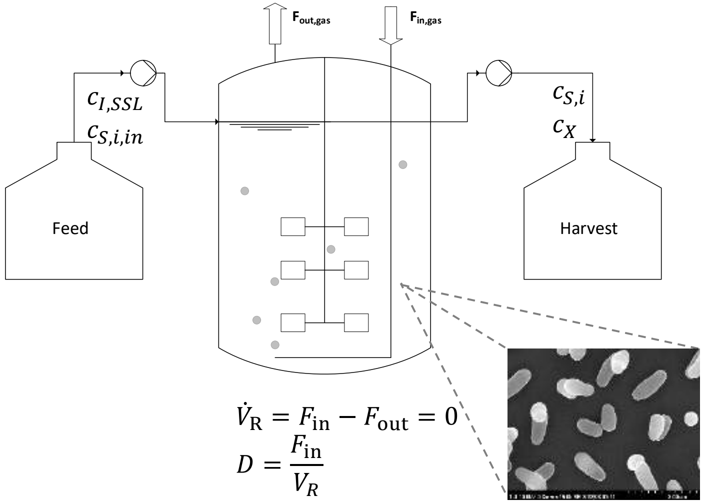
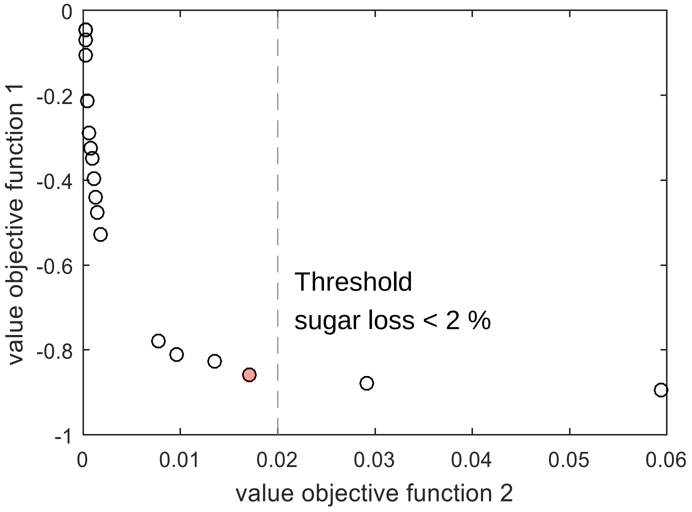


$$\dot{V}_R = F_{in} - F_{out} = 0$$
$$D = \frac{F_{in}}{V_R}$$

Pareto optimal solutions



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Unproductive region: washout

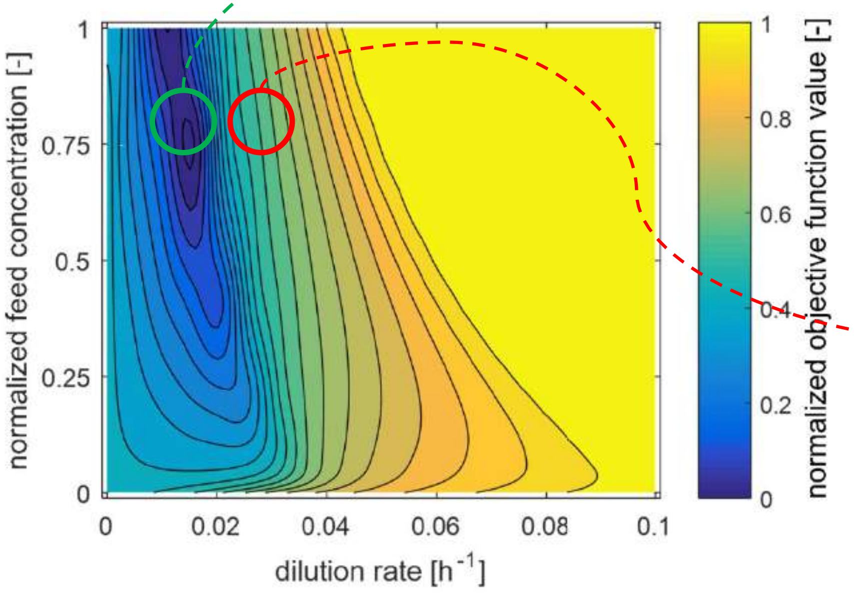
Targeted region: to be further analysed

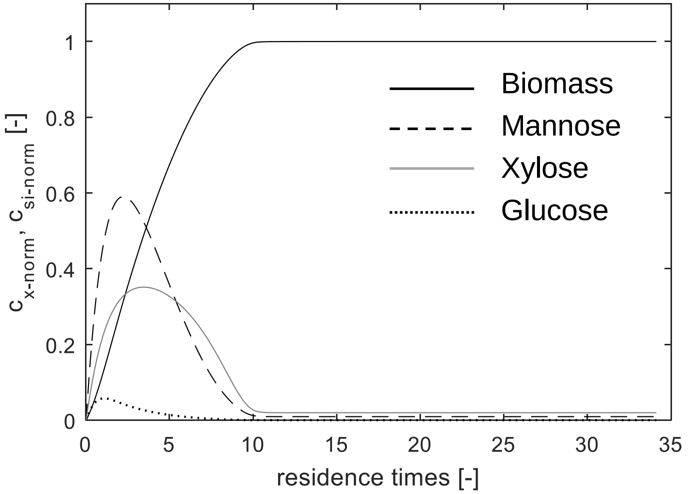
Model-based process design

- Analysis of operational points: small changes in dilution rate determine process efficiency

$$\min_{D, c_{I,SSL}} \; J_1 = -c_{X,\text{steady state}} D$$

$$\min_{D, c_{I,SSL}} \; J_2 = \frac{\sum_{i=1}^{3} c_{S,i,\text{steady state}}}{c_{S,1,\text{in}} + c_{S,2,\text{in}} + c_{S,3,\text{in}}}$$



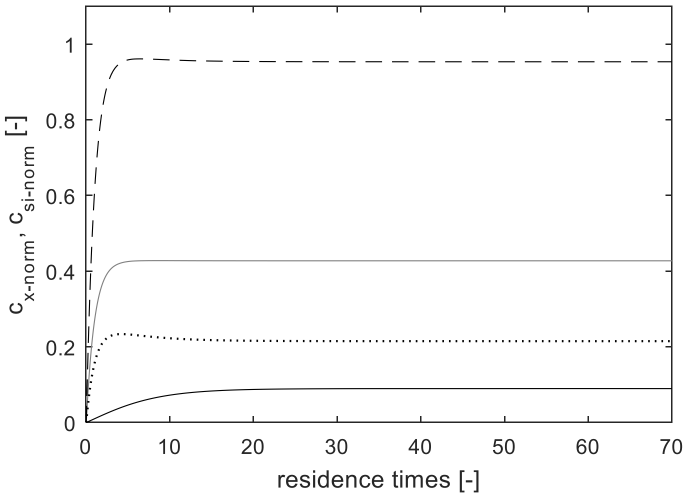


D = 0.013 h^{-1}

c_{feed} = 0.84

STY_{opt} = 0.86 g L^{-1} h^{-1}

$C_{sugar,residual}$ = 1.7 %



D = 0.035 h^{-1}

c_{feed} = 0.84

STY = 0.30 g L^{-1} h^{-1}

$C_{sugar,residual}$ = 91 %

Sinner et al., IFAC PapersOnLine 52-26 (2019)
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Model-based process design

- Process understanding based on parameter sensitivity analysis

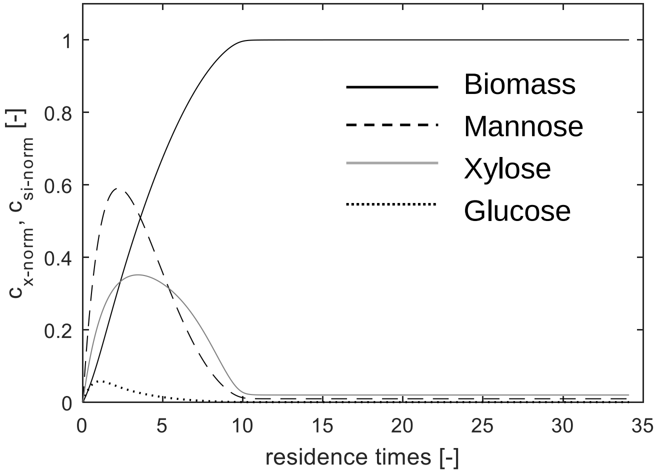
Optimized process

D = 0.013 h^{-1}

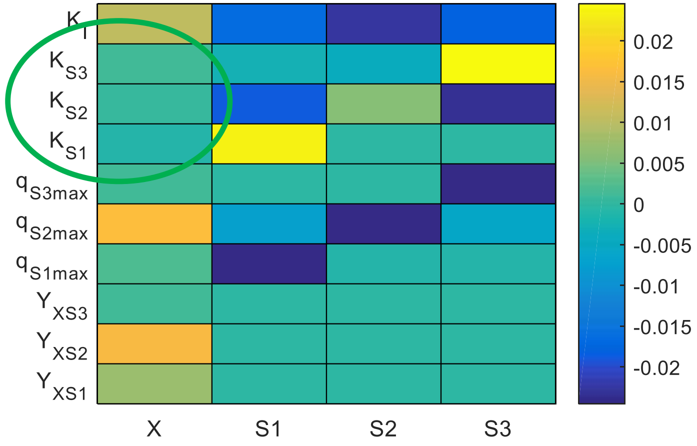
c_{feed} = 0.84

STY_{opt} = 0.86 g L^{-1} h^{-1}

$C_{sugar,residual}$ = 1.7 %



Sensitivity matrix



Biomass formation not inhibited by recipricocal sugar interaction

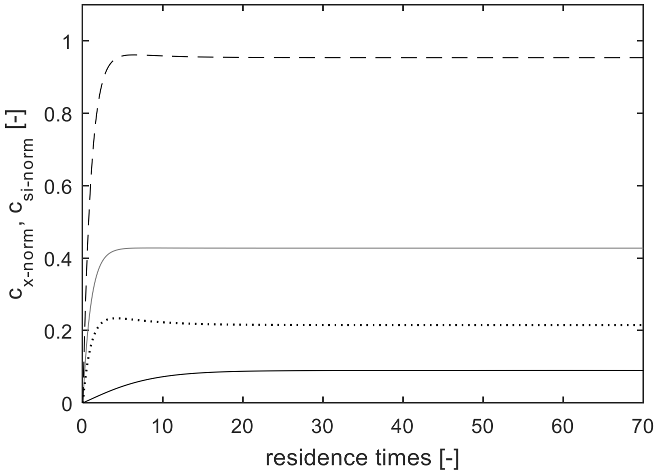
Unoptimized process

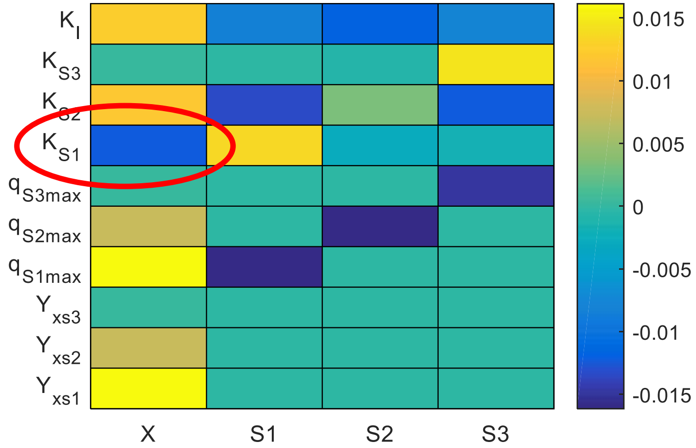
D = 0.035 h^{-1}

c_{feed} = 0.84

STY_{opt} = 0.30 g L^{-1} h^{-1}

$C_{sugar,residual}$ = 91 %





Biomass formation restricted by glucose inhibition effect

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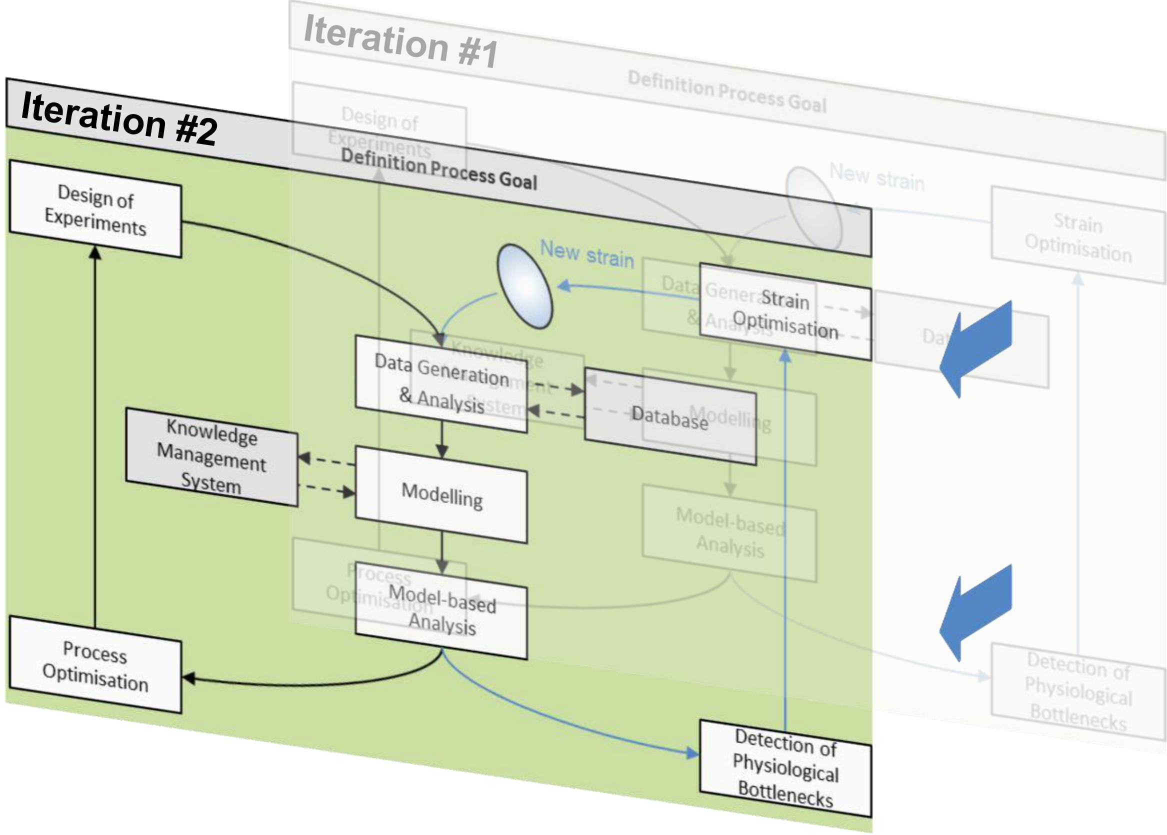
https://doi.org/10.1016/j.ifacol.2019.12.255

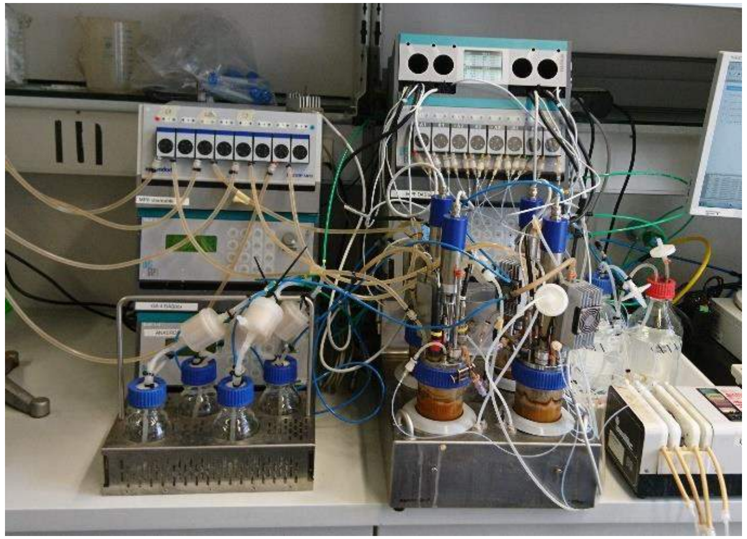
Bioprocess systems engineering

Iterative process development

- Characterisation of new strain variants
- Model adaption
- Process parameter optimization
- Experimental verification of *in-silico* optimized conditions



Strain characterisation with online monitoring

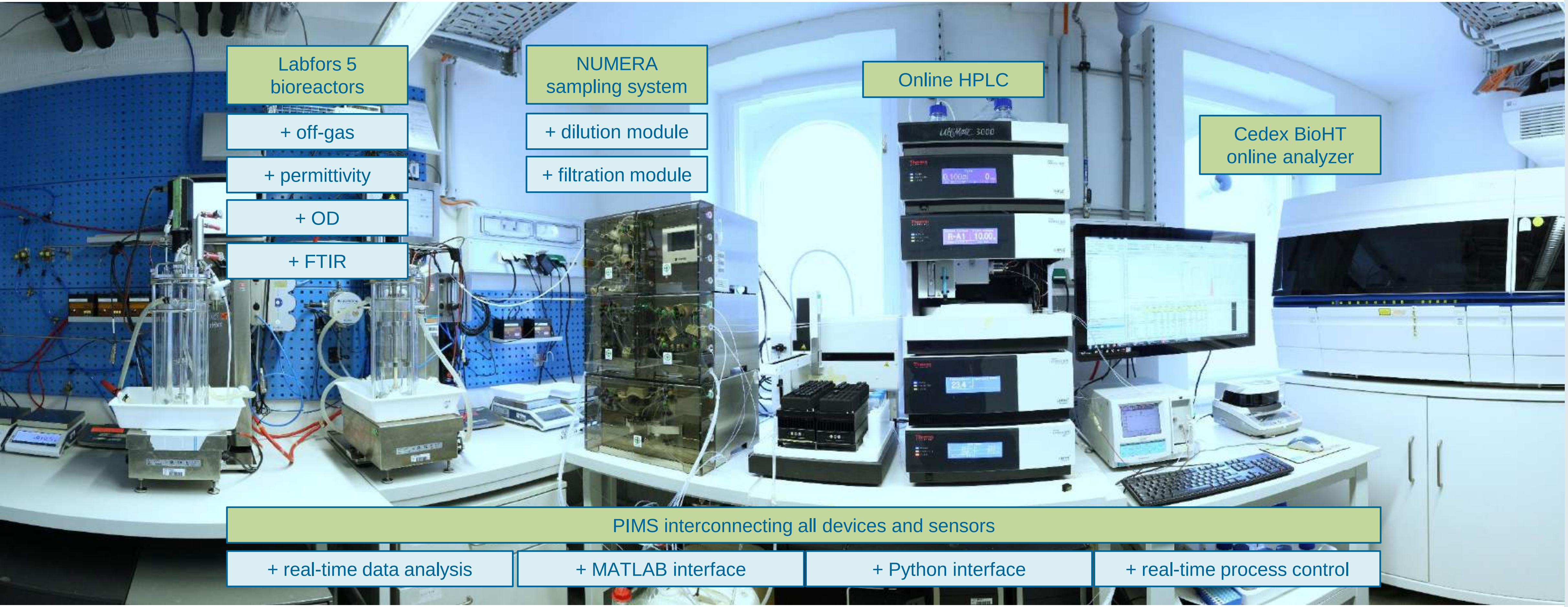


Implementation of continuous processes

Bioprocess systems engineering

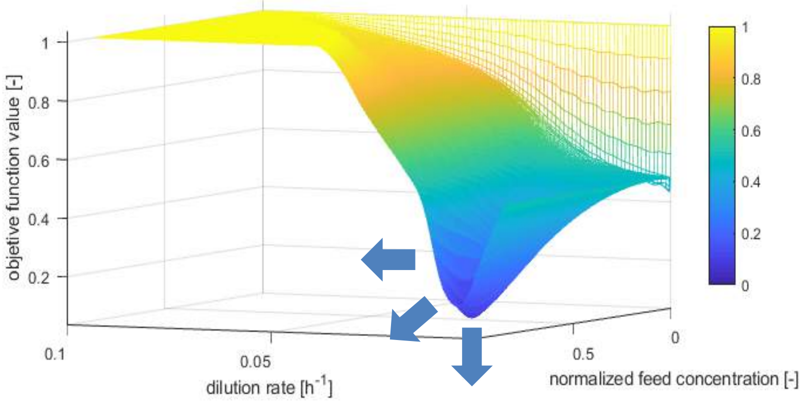
Process Analytical Technologies (PAT) Lab

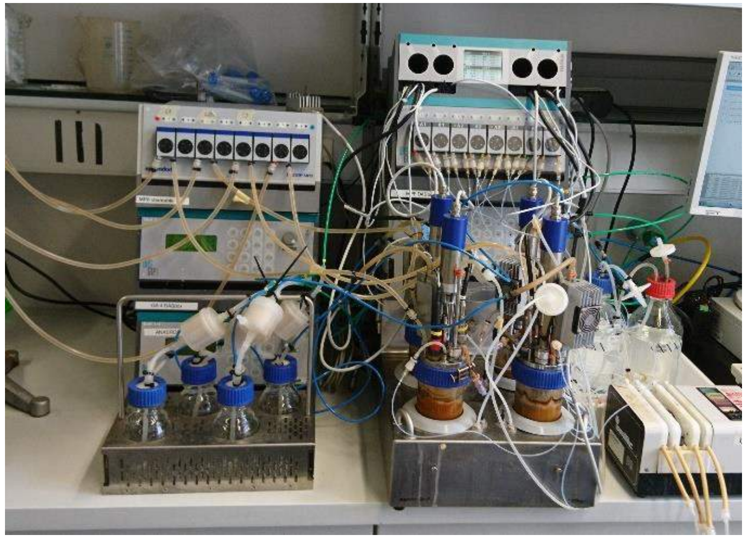
- Analysis of new production strains and bioprocesses on a fully automated & digitalized platform
- From online monitoring to model-based process design & control



Project achievements

- **Strain characterization on digitalized bioreactor platform**
- **Target oriented generation of a dynamic process model**
- **Model-based process design using multi-objective optimization**
- **Model-based detection of bottlenecks limiting the design space**
- **Implementation of a continuous process utilizing SSL**





Thank you!

TU Wien
ICEBE
Research Division Biochemical Engineering
Gumpendorferstrasse 1a / 166-4
A-1060 Wien
Austria

Email: christoph.herwig@tuwien.ac.at
Tel (Office): +43 1 58801 166400
Tel (Mobile): +43 676 47 37 217
Fax: +43 1 58801 166980

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Norwegian University of Science and Technology

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