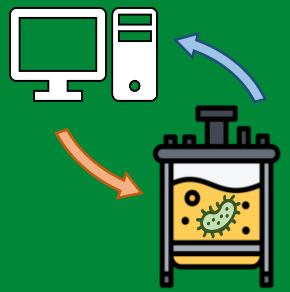
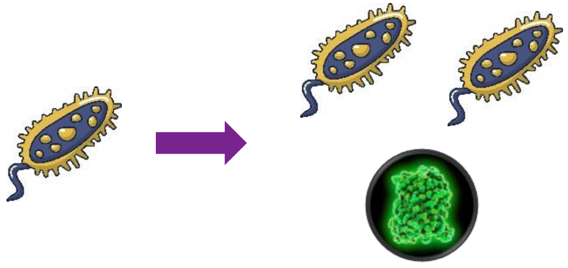


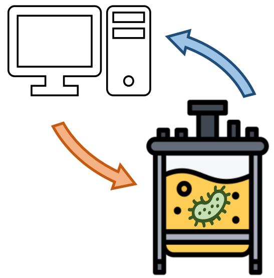
Philipp Pably, Pilot Plant / Chemical and Biochemical Engineering

MIMO control for recombinant protein production in a 2-feed microbial cultivation

Motivation

- Microbial bioprocesses are interesting process alternatives
  – Difficult to optimize
- Challenges for modelling & control
  – Complex, non-linear process dynamics
  – Non-steady state (batch operation)
  – Limited (online) measurements
- Opportunity for process improvements
  – Through process models & model-based control

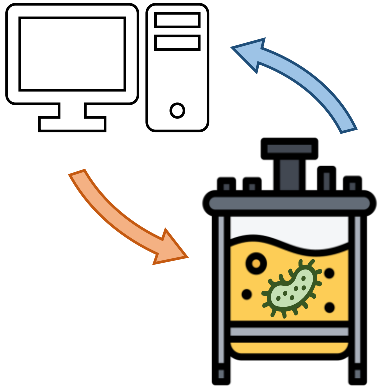




Case study: 2-feed system for recombinant protein production

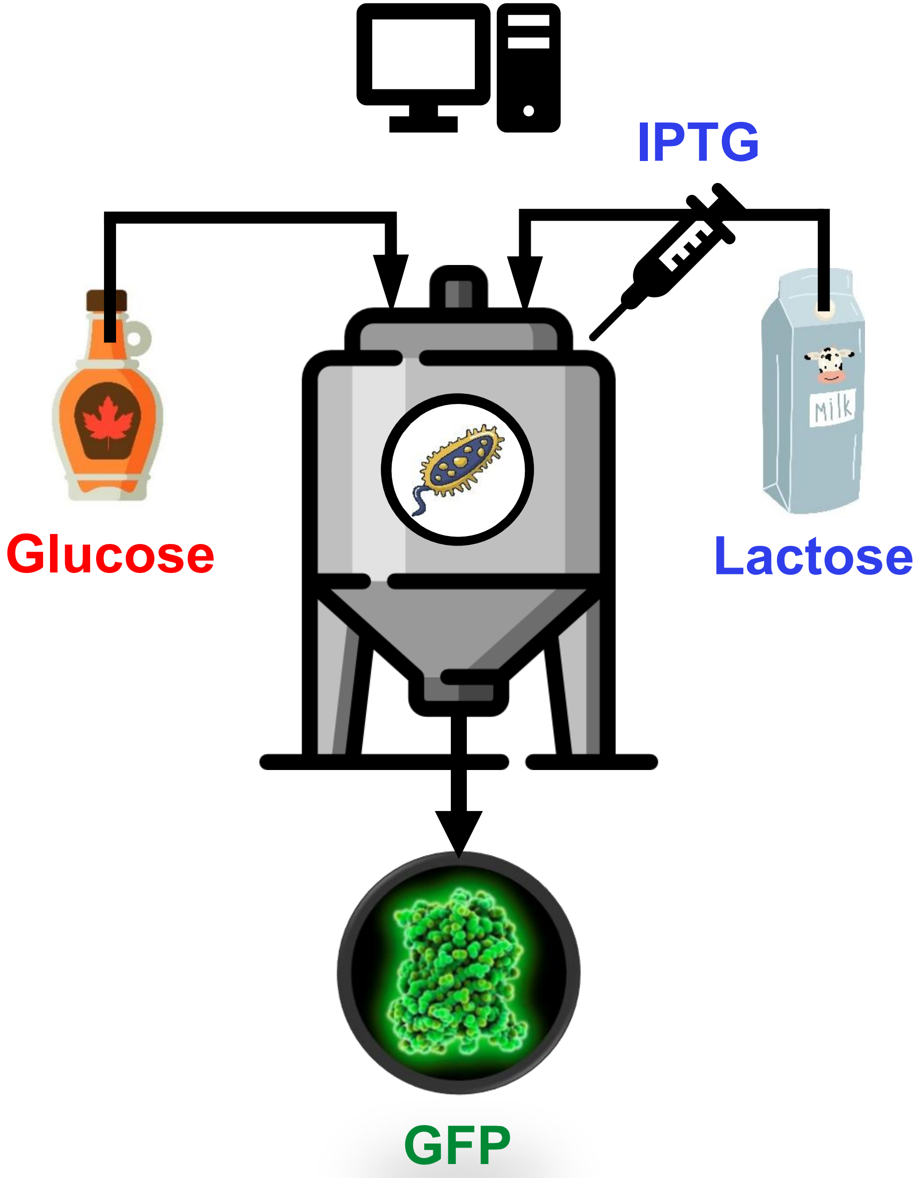
Workflow

1. Process description
2. Model development
3. Process optimization
4. Model-based control
5. Conclusions & Outlook



Process description

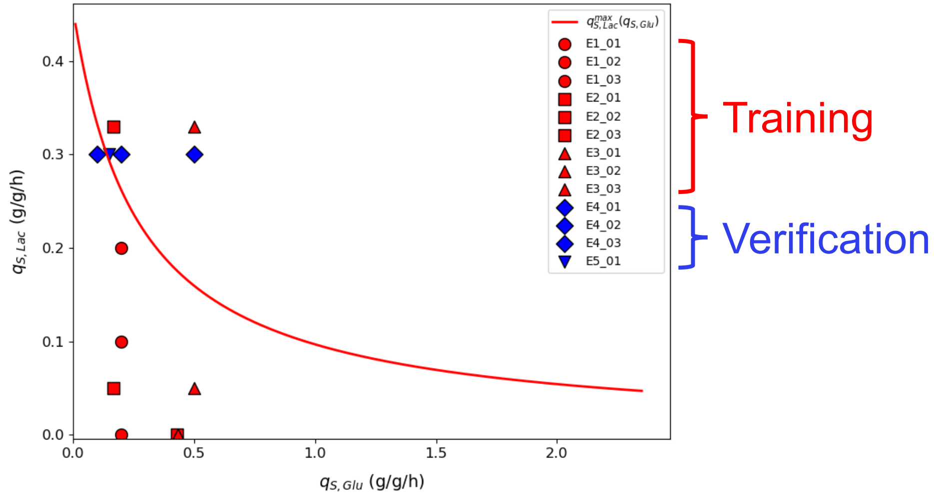
- *Escherichia coli* producing GFP
- Commonly one-shot induction as trigger
  – High expression, high stress on cells
- Alternative → Lactose as inducer
  – Strain fully digests additional substrate
- 2-feed *E. coli* cultivation
  – Possibility for tunable lactose/induction levels
  – Control of growth and productivity



Model development

- 2L-vessel bench-scale reactors (BSR)
  - pH, Temperature, DO control
  - Dynamic feed-setpoint experiments

- 5 dynamic experiments
  - 13 different setpoint combinations
  - 3/2 reactor split for training/verification data



Model development – Structure

- State-space model with mechanistic basis
  - Mass & volume balances
  - Feed inflows and sampling outflows
  - Focus on physiological variables

$$\frac{dx}{dt} = \frac{d}{dt}\begin{bmatrix} c_X \\ c_S \\ c_I \\ V_R \end{bmatrix}$$

- Substrate uptake with common Monod-type

$$q_S = q_{S,max} \cdot \frac{c_S}{c_S + K_S}$$

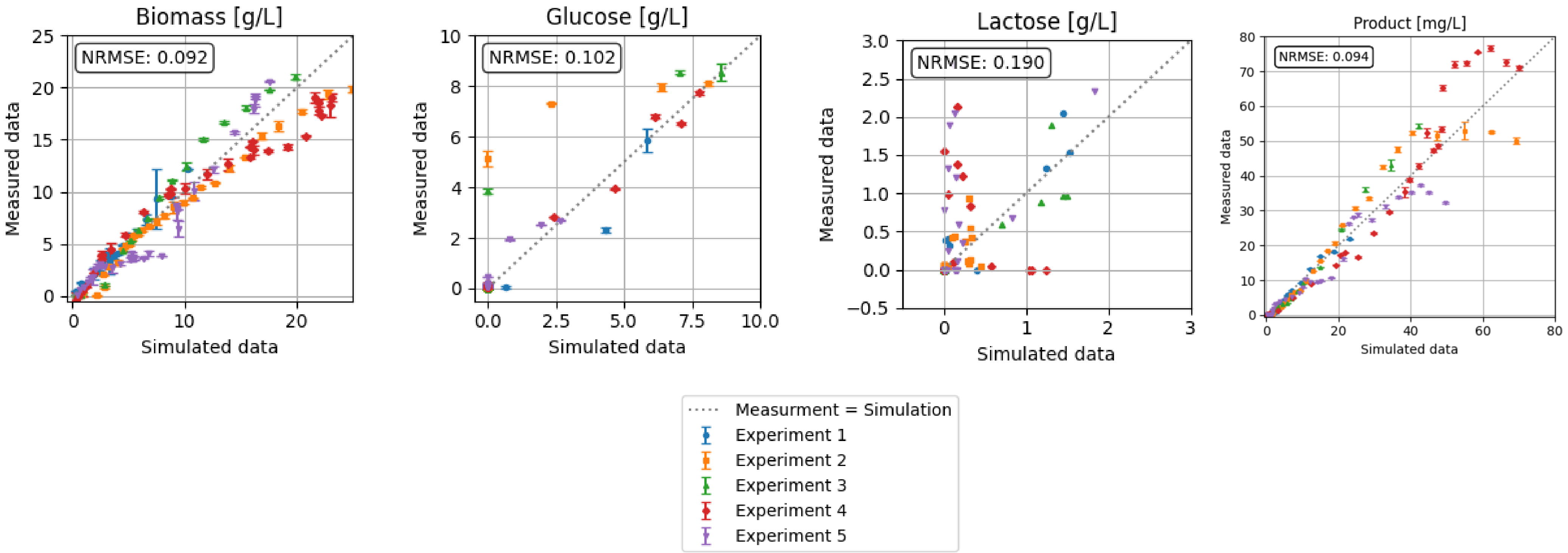
$$q_I = q_{I,max} \cdot \frac{c_I}{c_I + K_I} \cdot \frac{K_{IS}}{q_S + K_{IS}}$$

$$\mu = (q_S + q_I - q_m) \cdot Y_{\frac{X}{S}}$$

- Productivity with best AIC value from 12 kinetic formulations

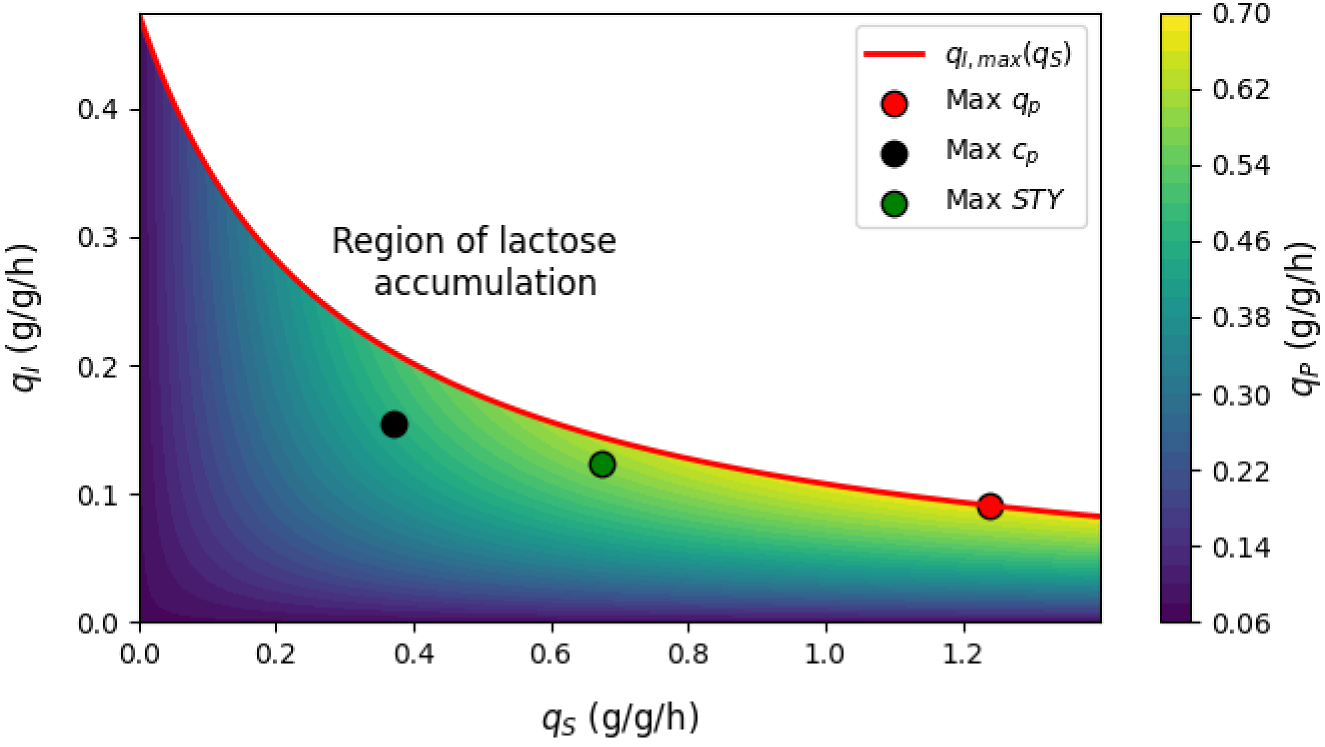
$$q_P = q_{P,max} \cdot \frac{q_I}{q_I + K_{PI}} \cdot \frac{q_S}{q_S + K_{PS}} + q_{P,base}$$

Model development – Fit

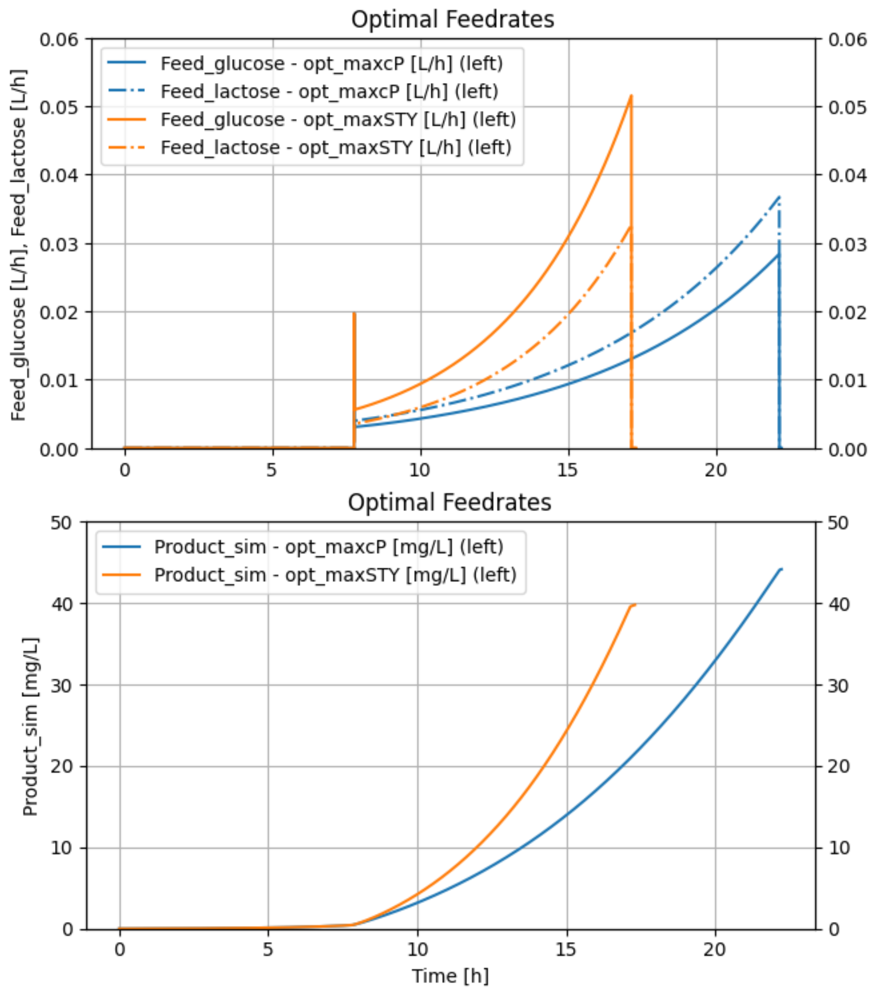


Process optimization

- Model describes q_P as function of $q_{Glucose}$ & $q_{Lactose}$
- Numerically optimized exponential feed setpoints



Boundary: 70g of total substrate fed



Process optimization – Verification experiments

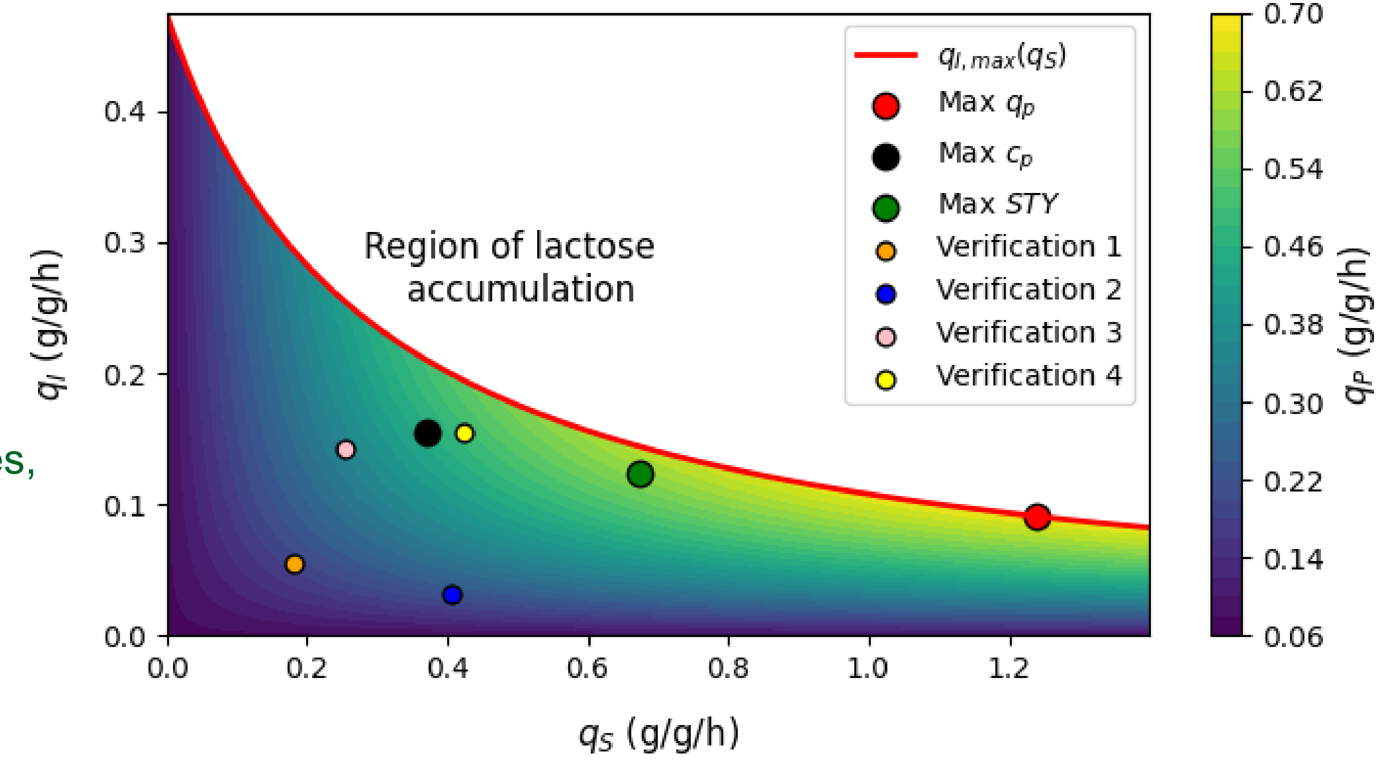
- 4 verification experiments
  - Test model accuracy and optimal setpoints
  - Difficulty of achieving soft-state setpoints for qS & ql

Pump calibration, weight balance resolution

$$q_S = \frac{F_{S,in} \cdot c_{S,in}}{c_x \cdot V_R}$$

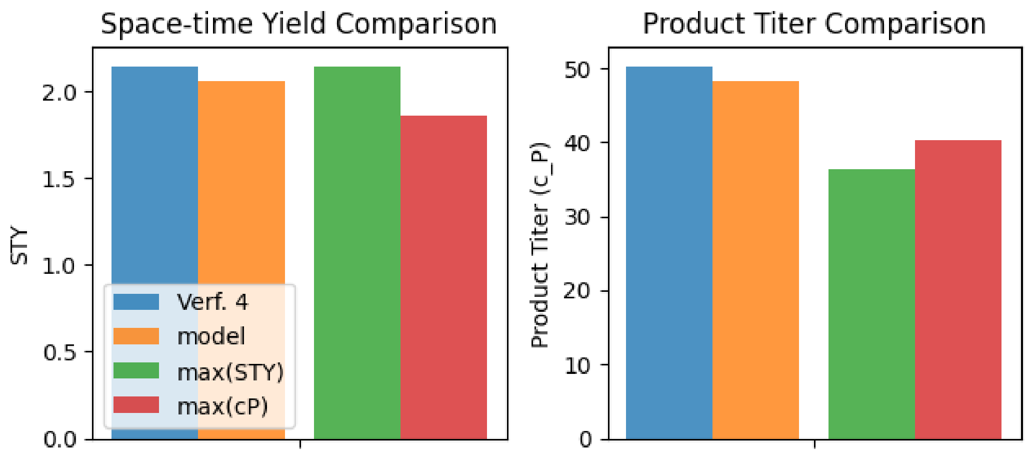
Estimation uncertainties, model parameters

Need for state estimation for improved control!



Process optimization – Verification results

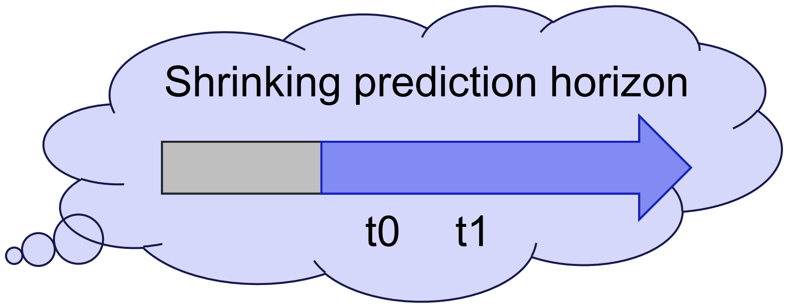
- Optimization goals max(STY) & max(c_P)
- Model & experiment metrics close – Verf.4 (max(c_P)) outperformed optimal design
  - – Experimental qS more dynamic than constant setpoint



- PID on pump with feedforward optimal setpoint improved process

How to obtain optimal setpoints when process deviates?

Model-based control - Structure



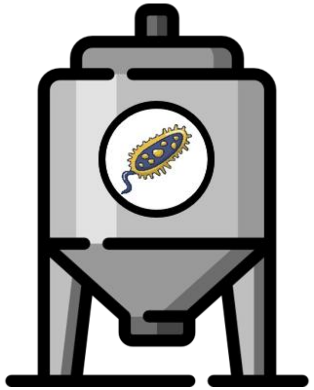
3) Economic MPC
- outer layer (slow t_S)
- Optimize setpoint trajectory
  - Maximize objective (c_P / STY)
  - Consider techn. limitations

Writes setpoints

Updates states with estimates

1) PID actuator controllers
- inner layer (fast t_S)
- Ensure setpoint tracking

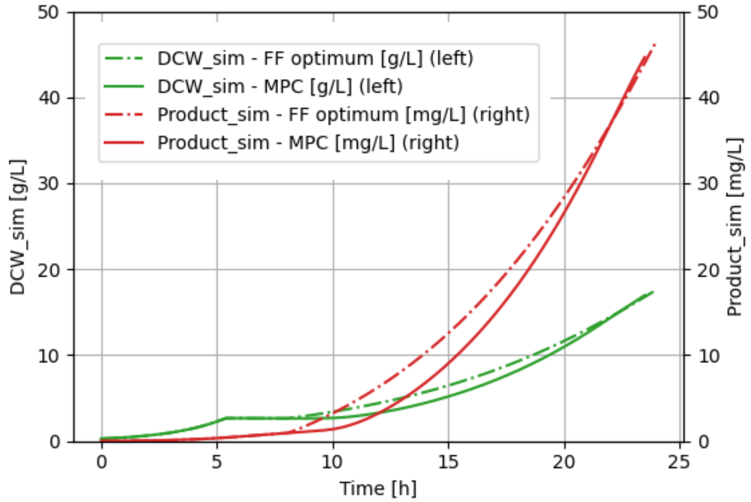
Control MVs



Measures

2) Soft-sensor / State estimator
- middle layer (fast t_S)
- State estimation
- Use online signals

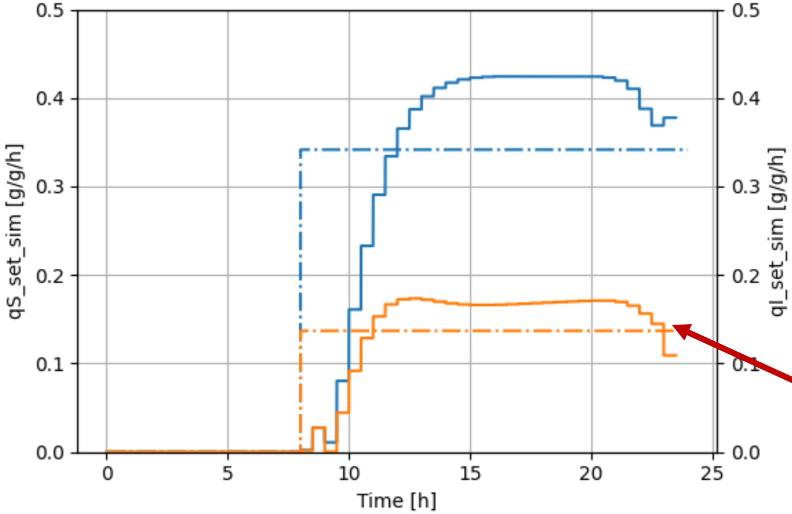
Simulations – Feedforward vs MPC



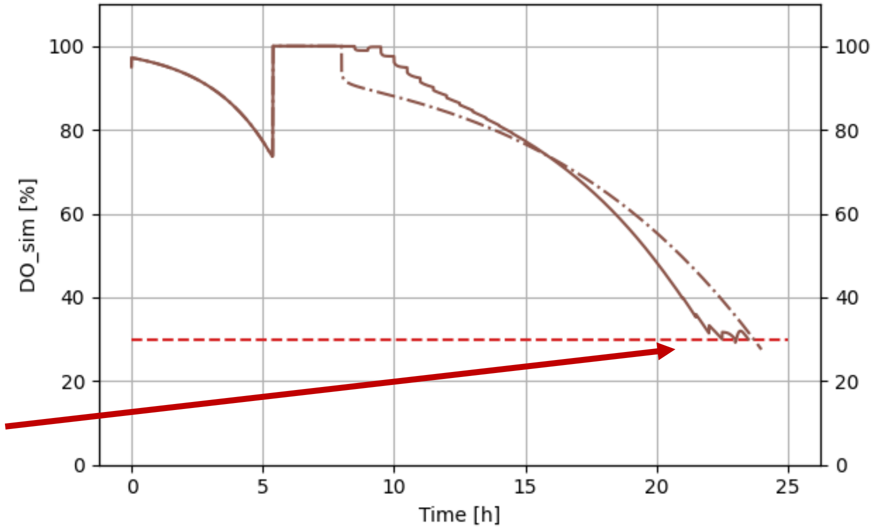
- Optimization problem
  - Lagrange term for smoothness $lterm = Q_S \cdot c_S + Q_I \cdot c_I$
  - Mayer term for terminal optimum

$$mterm = Q_S \cdot c_S + Q_I \cdot c_I - Q_{S,fed} \cdot S_{fed} - Q_P \cdot c_P$$

- Constraints for DO level and maximum S_{fed}

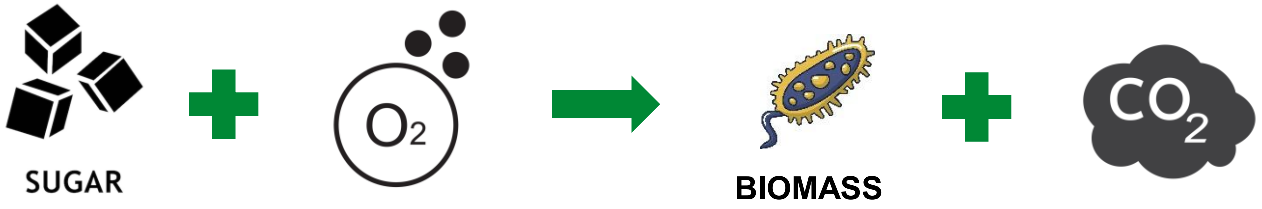


MPC considers limits in OTR and adjusts feeds!

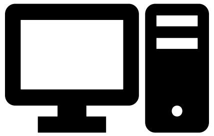


Model-based control - Potential

- **State-estimator** for process feedback
  - – Online offgas measurements
  - – Mechanistic relation to catabolism through stochiometry

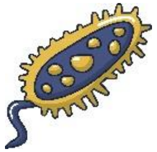


- MPC optimizes based on updated states
  - – Potential for online parameter re-estimation / process contraint estimation
  - – Optimal batch-end time detection



Conclusion & Outlook

- Model development
  – 2-feed cultivation model w experimental verification
  – Predicts growth & productivity



- Process optimization
  – Consistent with optimization goal & model
  – Feed-forward problems with qS / qI tracking

- Model-based control
  – PID setpoint tracking – MPC for optimal setpoint trajectory
- Outlook
  – Leverage offgas signal for state estimation
  – Experimental implementation

Acknowledgments

- Thank you to my supervisors!

Ass. Prof Julian Kager
DTU KT, PILOT

Prof Jakob K. Huusom
DTU KT, PROSYS

- Thank you to Eka & Alicia for the work!

- Thanks for funding!

novo nordisk foundation

Novo Nordisk Foundation, Start Package grants for faculty recruitment Q4 2022; assistant professor on bioprocess digitalization and control (NNF220C0081250)

Thank you for your attention!