Hes·so
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Master of Science HES-SO in Life Sciences

Digital Twin for growth rate prediction and control of *Lactobacillus rhasmnosus* cultures in fed-batch

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CHEMICAL DEVELOPMENT & PRODUCTION

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Introduction & Objectives

- *Lactobacillus rhamnosus* is widely used in biotechnology for functional foods and pharmaceuticals.
- Growth control in fed-batch cultures is essential to prevent unwanted metabolites and optimize yield.
- Traditional monitoring methods (e.g. TCD) are noisy and lack adaptability to process variations.
- Objective:
  - Implement an Artificial Neural Networks (ANN) as a digital twin of the biological system to predict growth rate (μ)
  - Integrate the ANN model into a closed-loop PI control system

Materials & Methods

- Microorganism: *Lactobacillus rhamnosus* LRH30
- Bioreactor: 1.8 L RALF equipped with sensor for: pH, Temperature, Dissolved Oxygen (DO), Total Cell Density (TCD), Gas analyser (Off-gas analysis: OUR, CER and RQ)

- ANN Model:
  - Implemented in Python using Keras 3
  - Training on normalized open-loop dataset (75% training, 12.5% validation, 12.5% test)

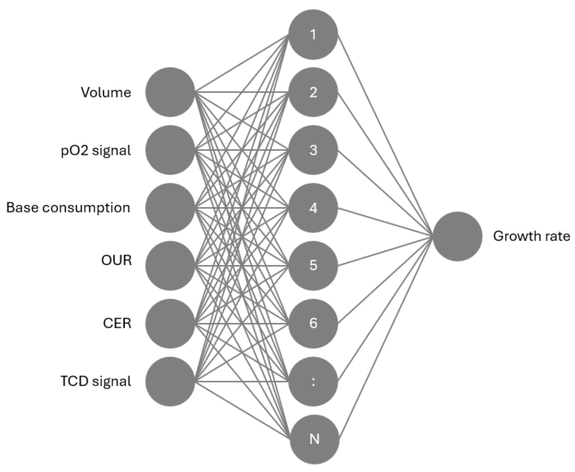

Figure 1. ANN Model geometry

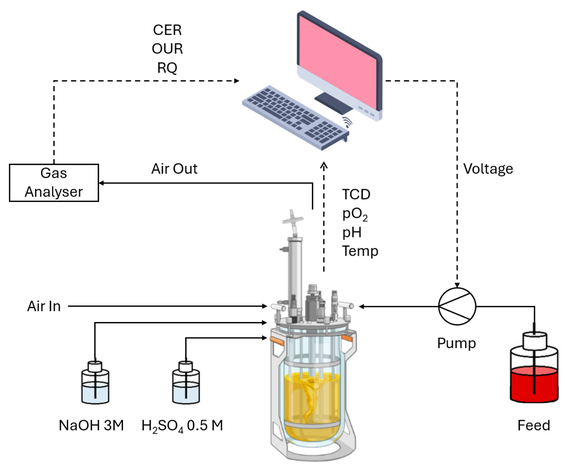

Figure 2. Reactor setup

- PI Control:
  - Implemented in LabView
  - Parameter tuning using Ziegler-Nichols method
  - Dynamic substrate feed regulation

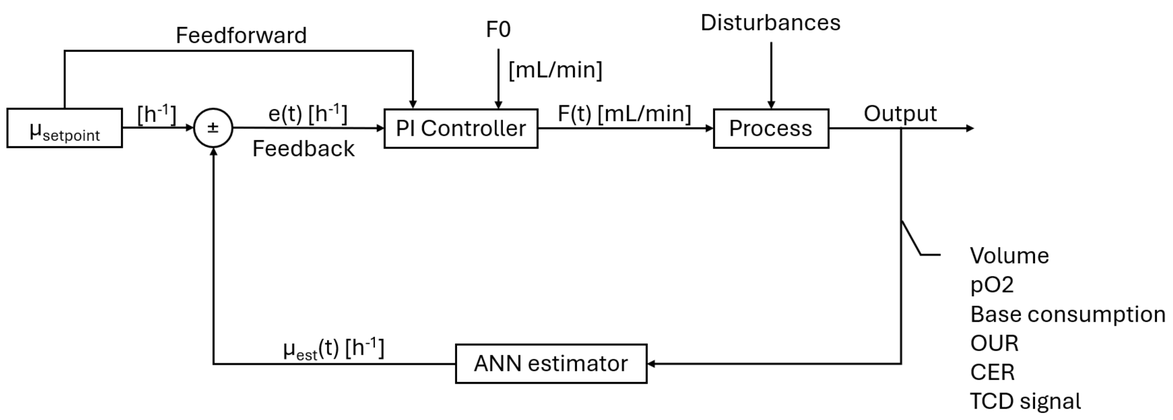

Figure 3. PI controller block diagram

Results

- ANN Model
  - RMSE = 0.073 h^{-1}
  - R^2 = 0.999
  - Carbon Emission Rate (CER) = most influential variable (SHAP analysis)
  - Better performances than the model developed by Sørensen et al. (DOI: 10.1016/j.crfs.2023.100593)

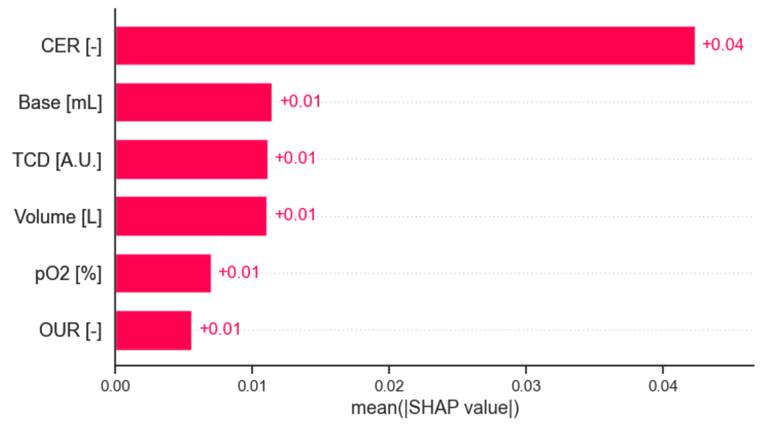

Figure 4. Model parameters influence

- Test with an exponential feed:
  - Less noise in signal compared to TCD probe
  - Less accuracy compared to

Entry	μ setpoint [h^{-1}]	SNR TCD Probe [-]	SNR ANN Model [-]
1	0.10	0.41	1.13
2	0.20	0.33	1.16
3	0.25	0.39	0.78
4	0.30	0.46	1.25

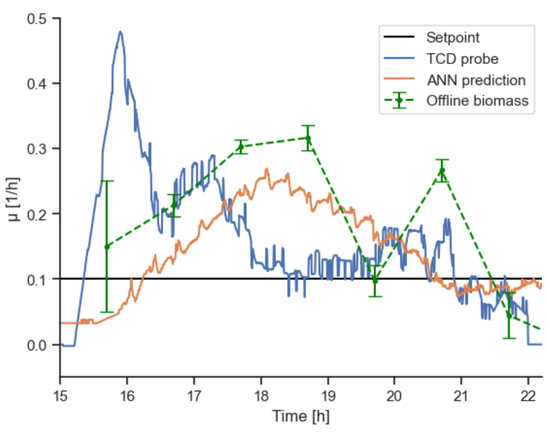

Figure 5. Growth rate profile for the test using an exponential feed, $\mu_{setpoint}$ = 0.25

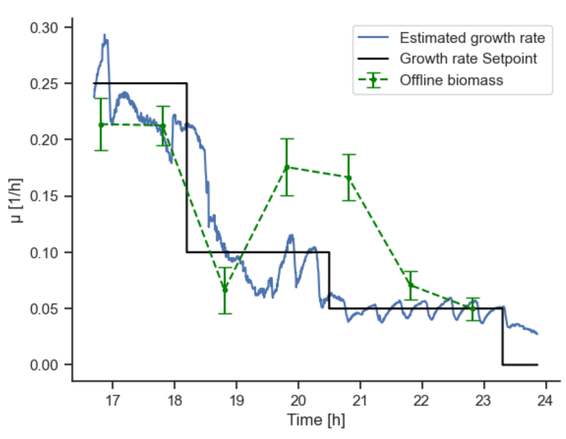

Figure 6. Growth rate profile for the test using the developed PI controller

- PI Controller Performance
  - Average control error 16.8% ± 5.41%
  - Reduced signal noise
  - Better control performances compared to Brignoli et al. (DOI: 10.3390/pr8060679)

Entry	μ setpoint [h^{-1}]	Mean Controller Error [%]	Overshoot [%]	Response Time [min]
1	0.25	14.8	12	12
2	0.10	24.2	40	48
3	0.05	11.4	20	18
Mean	-	16.8	24	26
Standard deviation	-	5.41	12	16

Conclusion

- A Digital Twin based controller improves growth rate control of *L. rhamnosus* in fed-batch processes
- PI control with ANN-based μ prediction ensures higher stability and reproducibility thanks to noise reduction
- Future developments
  - Increasing ANN complexity/Optimize ANN architecture
  - Expanding the prediction model to other microorganisms and bigger reactor scale