Hes·so
Haute Ecole Spécialisée de Suisse occidentale
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Master of Science HES-SO in Life Sciences

Real-Time Monitoring and Model Predicitve Control for Inclusion Body Fermentations

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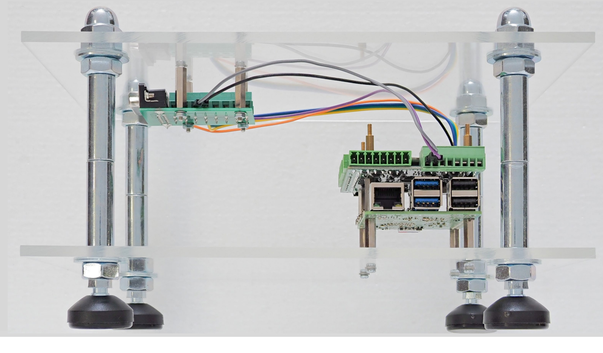

DESCRIPTION

Today microbial hosts and especially *E. coli* remains a preferred platform to produce simple recombination proteins due to its rapid growth and cost-effectiveness. Recombinant protein production in *E. coli* often leads to the formation of inclusion bodies (IBs) which are insoluble protein aggregates inside the cells. This study focused on controlling the IB fermentation process with nattokinase as a model protein. The feasibility of controlling the process using Model Predictive Control (MPC) and the calibration of the necessary models for real-time measurements was investigated. A custom closed-loop control infrastructure was designed and implemented into the existing fermentation set-up. For this a Raman spectrometer and a UV/VIS/NIR spectrometer were employed as sensors to test the feasibility of calibrating models for the real-time prediction of nattokinase, biomass, glucose and acetate via PLS modelling. A Raspberry Pi was employed to handle data transfer and run the MPC algorithm.

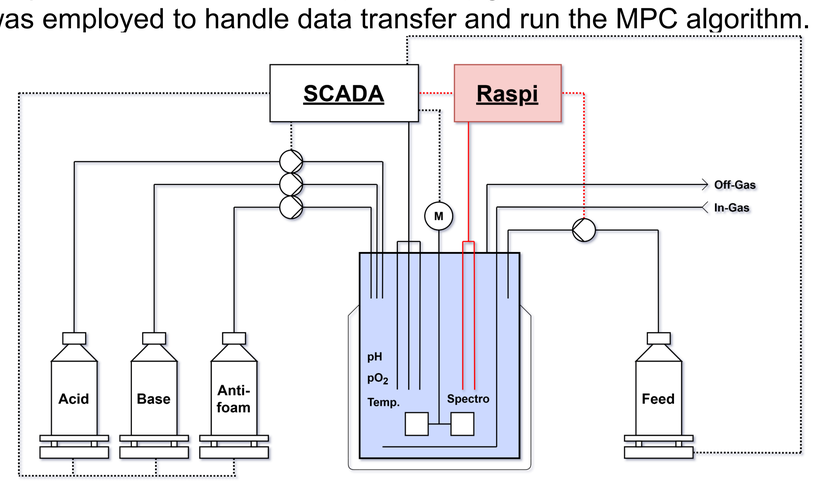

Fig 1: Schematic of the established MPC infrastructure

OBJECTIVES

The objective of this work was to implement and evaluate MPC for IB fed-batch fermentations using real-time in-line measurements and benchmark with the standard open-loop processes. This involved optimizing the cultivation medium, calibrating predictive models for glucose, biomass, nattokinase, and acetate, and integrating a Raman and a UV/VIS/NIR spectrometer for in-line data collection. The MPC controller was developed in Python and deployed to a Raspberry Pi 5. Multiple fermentations were conducted controlling the specific growth rate to test and refine the MPC algorithm, addressing issues and improving the process with each iteration. The final step was to analyze the data and compare MPC-controlled fermentations to open-loop results.

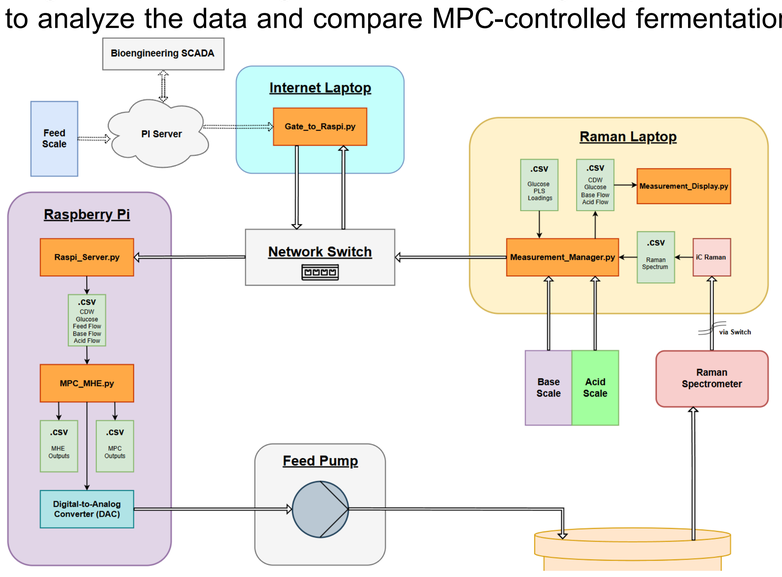

Fig 2: Overview of the established control infrastructure

RESULTS

The control infrastructure for MPC was successfully implement and tested. Four calibration fermentations were conducted to calibrate the sensors, namely the spectrometers.

Data from the Raman spectrometer was used to predict the glucose concentration for MPC. The best Partial Least Squares (PLS) model for glucose had an $RMSE_{CV}$ as low as 0.44 g/L. The UV/VIS/NIR data was less useful than Raman data for prediction of all modeled analytes.

PLS models for biomass were however deemed not good enough for MPC. Therefore, for the estimation of the biomass concentration as input for MPC, a correlation with the amount of added base for pH control was found and successfully applied.

Using these real-time inline measurement models, it was possible to control the specific growth rate using the MPC algorithm shown in Figure 3.

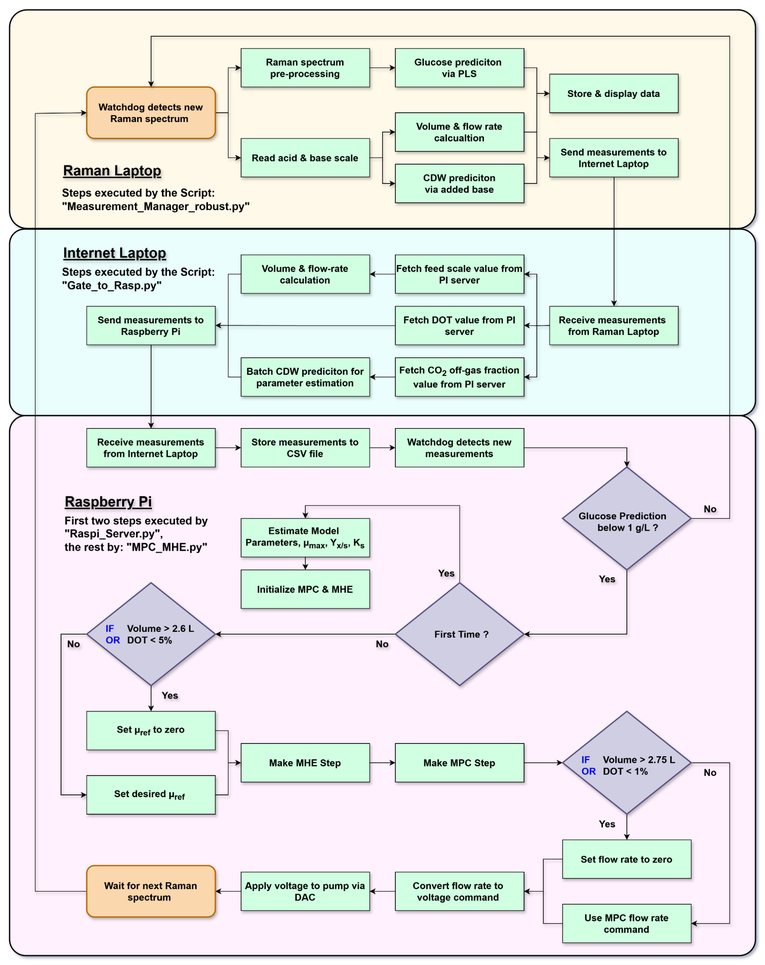

Fig 3: Flowchart of the established MPC algorithm

Using MPC, the classic open-loop approach was outperformed in the last and most optimized MPC fermentation in terms of achieved biomass concentration and volumetric nattokinase concentration as can be seen below. Also shown are diagrams of all parameters relevant to MPC from the last fermentation carried out with the latest version of the MPC algorithm.

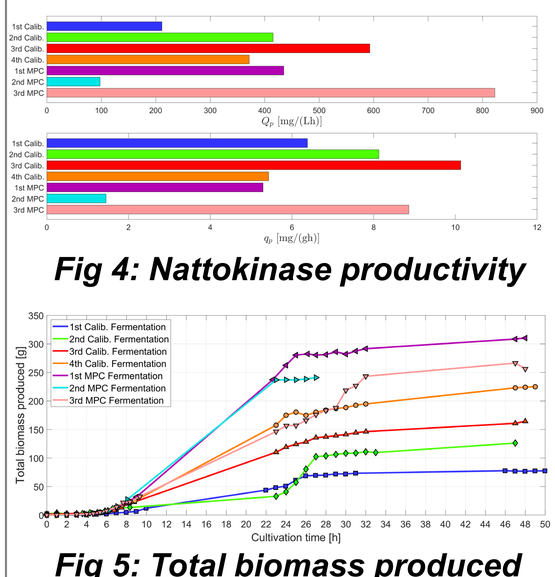

Fig 4: Nattokinase productivity

Fig 5: Total biomass produced

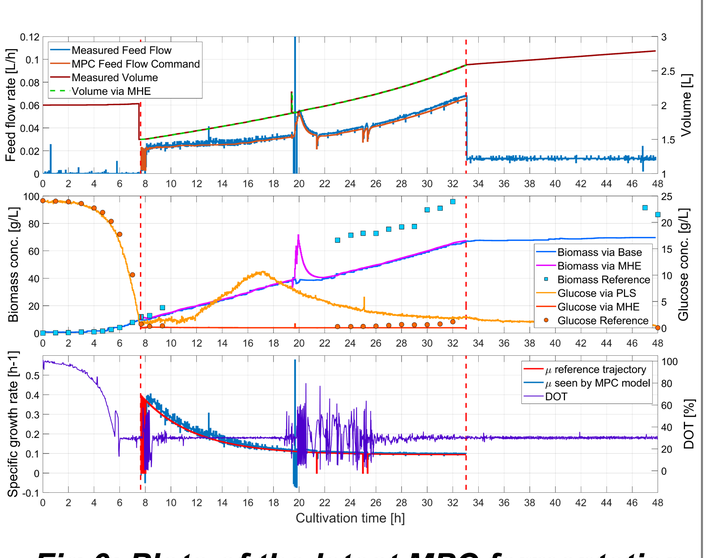

Fig 6: Plots of the latest MPC fermentation

CONCLUSION

This study successfully implemented and benchmarked MPC-controlled fed-batch fermentations against open-loop control for nattokinase production. Glucose prediction models were accurate but biomass and nattokinase predictions were unreliable due to turbulence and low signal strength. The MPC system demonstrated increasing control stability over three fermentations, outperforming open-loop control in biomass accumulation. Future work should focus on robust MPC to handle parameter uncertainties, refining acetate modelling, and investigating temperature reduction after induction to enhance protein production.

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