Parameter identification and sensitivity analysis of a dynamic model of *Rh. capsulatus* cultures

M. C. R. Nunes[1,*], L. Dewasme[1], M. Gilson[2], B. Leroy[2], A. Vande Wouwer[1]

[1] Systems, Estimation, Control and Optimization (SECO), University of Mons, 31 Boulevard Dolez, 7000, Mons, Belgium.
[2] Laboratory of Proteomics and Microbiology, University of Mons, 6 Avenue du Champs de Mars, 7000, Mons, Belgium.
*Corresponding author: matheus.camargoromanonunes@umons.ac.be

Keywords: mathematical modeling · parameter estimation · biotechnology · photorefineries · purple bacteria

Introduction

Despite recent progress in purple non-sulfur bacteria (PNSB) technology, further research regarding process design and efficiency (Capson-Tojo et al., 2020) is required to realize its full potential. Mathematical modeling emerges as a key asset, enhancing process comprehension and serving as a tool for model-based control and optimization. The relevance of this topic is justified by the substantial number of contributions focused on modeling PNSB, notably Puyol et al. (2017) and Cabecas Segura et al. (2022). Although the complexity of the former may limit its use for control/optimization, the latter is likely to be a suitable alternative. The fact that it is directed to another PNSB strain with different carbon sources distinguishes it from our study. In this work, we propose a macroscopic model to predict *Rh. capsulatus* growth on sucrose, fructose, and glucose. The model is obtained by employing measurements from batch experiments on synthetic conditions that mimic PNSB growth on molasses for microbial protein production. Considering the need to optimize production conditions, we aim to provide a model to predict the dynamic behavior of PNSB cultures while having potential control and optimization applications in mind. To this end, we evaluate the fit quality and parameter sensitivity.

Materials and Methods

The mathematical model is derived following a procedure inspired by Bastin and Dochain (1990). Based on prior knowledge, the biological reactions and Contois-like reaction rates are described in Tab.1, as well as the ordinary differential equations representing the evolution of each component, which are obtained by applying mass balance. Fructose and glucose are directly consumed, and sucrose is assumed to be hydrolyzed by the cells at a reaction rate that follows the Michaelis-Menten equation.

Tab. 1. Model description.

Macro-reactions	Rate equations	Mass balances
$suc \xrightarrow{\varphi_h} fru + glu$ $Y_{fru}\, fru \xrightarrow{\varphi_1} X$ $Y_{glu}\, glu \xrightarrow{\varphi_2} X$	$\varphi_1 = \mu_{max1} X \frac{fru}{K_1 X + fru}$ $\varphi_2 = \mu_{max2} X \frac{glu}{K_2 X + glu}$ $\varphi_h = \frac{V_h\, suc}{K_h + suc}$	$dX/dt = \varphi_1 + \varphi_2$ $dfru/dt = -Y_1 \varphi_1 + \varphi_h$ $dglu/dt = -Y_2 \varphi_2 + \varphi_h$ $dsuc/dt = -\varphi_h$

where X is the biomass optical density (OD) and fru, glu, and suc are, respectively, the concentrations of fructose, glucose, and sucrose in mmol/L. φ_1 and φ_2 are the respective reaction rates for the fructose and glucose consumptions, while φ_h is the hydrolysis rate. The parameter sets $[\mu_{max1}, K_1, Y_1]$ and $[\mu_{max2}, K_2, Y_3]$ contain the corresponding specific growth rate (h^{-1}), half-saturation constant (mmol/L), and yield coefficient (mmol/OD.L) for fructose and glucose, respectively. Lastly, V_h is the maximum hydrolysis rate (mmol/L.h) and K_h(mmol/L) is the Michaelis-Menten half-saturation constant.

Parameter identification and sensitivity analysis comparable to Fekih-Salem et al. (2019) are performed on a set of 4 batch experiments with various initial biomass and nutrient source concentrations, as well as constant nitrogen (provided in excess) levels and artificial light intensity. Parameter estimation considers the minimization of a weighted least-squares criterion describing the distance between the experimental data and the model predictions. Parametric sensitivities are also computed to build the Fisher Information Matrix (FIM). Its inverse form is then employed to obtain an estimation of the parameter estimation error covariance matrix. Confidence intervals (CI) at a 95% are retrieved from the diagonal of this matrix for each parameter.

Given that knowledge about the metabolic network is subject to ongoing research and thus not entirely comprehended, various scenarios, such as inhibition terms and modified rate structures, are tested to select the best model. To assess model fitting performance, we adopt a root-mean-square (RMSE) criterion.

Results and discussion

Model predictions' results and the experimental data are shown in Fig.1. The model is visually consistent with the measurements, which is confirmed by the low RMSE criteria values presented in Tab.2. Although the calculated CI's of the estimated parameters are slightly larger than desired, the model predictions are satisfactory, considering the limited amount of data used to identify such a complex bioprocess. Indeed, further improvement is possible by obtaining additional data, but the potential benefit should be carefully evaluated, with time and cost as decision factors. Moreover, implementing a robust control strategy could also address the parametric uncertainty. In conclusion, the results confirm the proposed model's predictive accuracy and suitability for future advanced control applications.

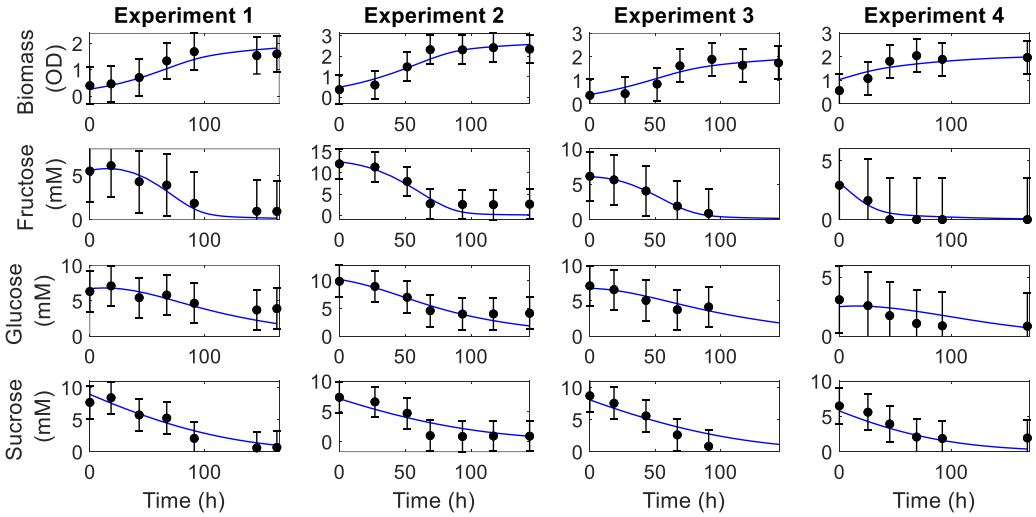

Fig. 1. Blue continuous lines represent the model predictions for the concentrations of biomass, fructose, glucose, and sucrose and experimental data is indicated by black dots. The bars correspond to *a posteriori* calculations of the 95% confidence intervals.

Tab. 2. Parameter identification and sensitivity analysis results.

Stoichiometric and kinetic parameters (%CI)	RMSE results
$Y_1 = 11.2\ (113\%); Y_2 = 34.9\ (433\%)$ $K_1 = 1.46\ (182\%); K_2 = 13.5\ (165\%);$ $\mu_{max1} = 0.0185\ (126\%); \mu_{max2} = 0.0100\ (440\%);$ $V_h = 0.129\ (145\%);\ K_h = 5.99\ (259\%)$	$RMSE_X = 0.232$ $RMSE_{fru} = 0.878$ $RMSE_{glu} = 0.967$ $RMSE_{suc} = 0.987$

Acknowledgments

The research leading to these results has been funded by the Public Service of Wallonia (Economy, Employment and Research), under the FoodWal agreement n°2210182 from the Win4Excellence project of the Wallonia Recovery Plan.

References

Bastin, G. and Dochain, D. (1990). On-Line Estimation and Adaptive Control of Bioreactors. Volume 1 of Process Measurement and Control, Elsevier: Amsterdam.

Capson-Tojo, G., Batstone, D.J., Grassino, M., Vlaeminck, S.E., Puyol, D., Verstraete, W., Kleerebezem, R., Oehmen, A., Ghimire, A., Pikaar, I., et al. (2020). Purple phototrophic bacteria for resource recovery: Challenges and opportunities. Biotechnology advances, **43**, 107567.

Cabecas Segura P., De Meur Q., Alloul A., Tanghe A., Onderwater R., Vlaeminck S.E., Vande Wouwer A., Wattiez R., Dewasme L., Leroy B. (2022) Preferential photoassimilation of volatile fatty acids by purple non-sulfur bacteria: Experimental kinetics and dynamic modelling. Biochem. Eng. J. **186**, 108547.

Fekih-Salem R., Dewasme L., Castro C.C., Nobre C., Hantson A.L., Vande Wouwer A. (2019) Sensitivity analysis and reduction of a dynamic model of a bioproduction of fructo-oligosaccharides. Bioprocess Biosyst. Eng. **42**, 1793–1808.

Puyol D., Barry E., Hülsen T., Batstone D. (2017) A mechanistic model for anaerobic phototrophs in domestic wastewater applications: Photo-anaerobic model (PAnM). Water Res. **116**, 241–253.