Available online at www.sciencedirect.com

ScienceDirect

IFAC PapersOnLine 55-7 (2022) 762–767

A Simulation Study on Model-Based Optimization of Intracellular Trehalose Accumulation in *Saccharomyces cerevisiae* Fed-Batch Cultures ⋆

Mihaela Sbarciog * **Antoine Huet** * **Philippe Bogaerts** *

* *3BIO-BioControl, Université Libre de Bruxelles, CP 165/61, 50, Av. F.-D. Roosevelt, B-1050 Brussels, Belgium (e-mail: mihaela.iuliana.sbarciog@ulb.be; antoine.huet@ulb.be; philippe.bogaerts@ulb.be).*

Abstract: Trehalose is a reserve carbohydrate, which occurs naturally in yeast and acts as a stress protectant for cells. Due to its features, trehalose is used in many industries (food, biopharmaceuticals, cosmetics). Its production/accumulation may be triggered and enhanced by the culture operating conditions, among which the feed flow rate and the start of the starvation period play the most important role. This paper presents a simulation study based on a novel model validated with experimental data, highlighting the interplay between trehalose maximization and biomass maximization. Several single-objective and multi-objective constrained optimization problems are solved using the control vector parameterization approach. It is shown that maximizing either the trehalose concentration or the biomass amount leads to solutions which are not appealing in practice. The best solution is obtained by maximizing trehalose concentration with a lower bound on the biomass amount. A comparison of the optimal solution to experimental data reveals that, although approximately the same amount of biomass can be obtained with different feeding profiles and starvation times, the maximization of trehalose concentration requires feeding most of the culture medium in the first part of the experiment while starting the nitrogen starvation at an early stage.

Keywords: *Saccharomyces cerevisiae*, trehalose, dynamical model, process optimization, control vector parameterization, stress protectant

1. INTRODUCTION

Trehalose is a disaccharide formed of two glucose molecules which is naturally synthesized within yeasts as well as insects and plants. Over the years, this sugar drew the attention of several industries such as the agro-food, the pharmaceutical and the cosmetics industries (Ohtake and Wang, 2011).

In the baker's yeast industry, understanding and controlling *Saccharomyces cerevisae*'s intracellular accumulation of trehalose has become of utmost importance. During the various phases leading to dry yeast, the yeast cells are facing several stresses linked to the culture medium (hypertonicity, potentially toxic levels of ethanol) and to the mechanical process (yeast drying) leading to increased mortality rates. Moreover, the dried cells must survive the conservation time span and still recover their metabolic activities once they are activated by the baker. The intracellular accumulation of trehalose in yeast has been proven to be of crucial importance for cells resistance to various stresses. Not only is trehalose a carbon and energy reserve, but it also helps maintaining the structural integrity of the cell in stressful conditions generated by the cell's environment (Tapia and Koshland, 2014; Saini et al., 2018). Trehalose is, therefore, often referred to as a stress protectant. In fact, Wiemken (1990) argues that trehalose is more a stress protectant rather than a carbohydrate reserve.

Industrial production of *S. cerevisiae* is usually performed in fed-batch bioreactors. However, the art of selecting the best culture conditions to optimize the quantity and quality of commercial baker's yeast is not an easy task and generally results in a long and tedious trial and error process. This can be overcome by performing model-based process optimization (Richelle and Bogaerts, 2014; Mahmoodi and Nassireslami, 2021). Models simulating the growth of *S. cerevisiae* include generally the growth of cells on glucose and the overflow metabolism (Sonnleitner and Käppeli, 1986). Models predicting trehalose accumulation in yeast are scarce, hence, model-based optimization studies regarding trehalose accumulation are not available.

On the contrary, maximizing the amount of cells at the end of the culture, while minimizing the amount of ethanol seems to be the most common criterion for optimization of yeast cultures. Numerous studies have been published, which consider the control or optimization of the culture

⋆ This research has been funded by the Wallonia Region (SPW Recherche) and supported by Wagralim, the agri-food innovation cluster in Wallonia Region, within the framework of the WA-SuNuP project.

10.1016/j.ifacol.2022.07.536

conditions in view of improving the quantity and quality of biomass. Depending on the approach employed, these studies can be roughly classified in three groups: i) direct optimization using dynamic programming (Berber et al., 1998), genetic algorithms (Yüzgeç et al., 2009), control vector parameterization (Atasoy et al., 2013); ii) control of ethanol concentration at a critical level (Valentinotti et al., 2003; Renard et al., 2006); iii) control/maximization of biomass growth rate (Picó et al., 2009).

In view of trehalose importance for various industries, maximization of trehalose accumulation during the growth of *S.cerevisiae* is a sensitive and worth investigating research topic, with high impact. This paper presents a simulation study, which aims to highlight the interplay between the maximization of trehalose accumulation in yeast and the maximization of biomass, which represents also an important technological optimization problem. The study is performed based on a recent model identified from experimental data of *S.cerevisiae* fed-batch cultures (Huet et al., 2022), which extends the previously proposed model of yeast growth with coordinated uptake of glucose and ammonium (Richelle et al., 2014) to account for storage and mobilization of trehalose. The paper is organized as follows. Section 2 describes the process and introduces the macroscopic reaction scheme employed in the modeling. Section 3 details the macroscopic dynamical model (Huet et al., 2022) used in this study. The model-based optimization is discussed in Section 4, starting with the introduction of the control vector parameterization approach employed and detailing the investigated optimization problems. Conclusions are drawn in Section 5.

2. PROCESS DESCRIPTION AND MACROSCOPIC REACTION SCHEME

High yeast cell densities are achieved in a bioreactor operated in fed-batch mode, where nutrients are supplied continuously, while the produced cells, unused nutrients and conversion products are not removed before the end of the operation. The most common model used to describe yeast growth assumes that the cells need only a carbon source (typically glucose) for growth and includes the overflow metabolism as described by Sonnleitner and Käppeli (1986) to account for the Crabtree effect. However, aside the carbon source a nitrogen source (ammonium) is also required as nitrogen plays an important role in microorganisms growth and the activation of the cellular metabolism, since it is a main component of proteins and nucleic acids. Hence, the model employed in this work considers the coordinated uptake of glucose and ammonium and characterizes the storage and mobilization of trehalose. In fully aerobic conditions, the model relies on the reaction network (Richelle et al., 2014; Huet et al., 2022)

$$\begin{aligned} G &\xrightarrow{r_1} k_1 X \\ G &\xrightarrow{r_2} k_2 X + k_4 E + k_7 A \\ E &\xrightarrow{r_3} k_3 X \\ N + A &\xrightarrow{r_4} k_8 X \\ G_{int} &\underset{r_{mT}}{\overset{r_{sT}}{\rightleftharpoons}} \frac{1}{2} T \end{aligned} \tag{1}$$

where X, G, N, E, A, G_{int} and T respectively denote the biomass, extracellular glucose, ammonium, ethanol, intracellular α-ketoglutarate, intracellular glucose and intracellular trehalose. $k_1 \dots k_8$ represent the pseudo-stoichiometric coefficients. The reactions included in the network (1) express: i) biomass growth on glucose through respiration; ii) biomass growth on glucose through fermentation, with ethanol production and α-ketoglutarate accumulation; iii) biomass growth on ethanol through respiration, which is only possible if the global glucose uptake is inferior to the maximum respiratory capacity and occurs only in the presence of ethanol; iv) formation of biomass on ammonium as well as coordinated consumption of α-ketoglutarate, therefore boosting the fermentation as α-ketoglutarate is considered an inhibitor of the fermentation; v) storage of trehalose by consumption of the intracellular glucose and trehalose mobilization as release of the intracellular glucose.

3. MACROSCOPIC DYNAMICAL MODEL

The system dynamics are described by the mass balance equations

$$\begin{aligned} \frac{dX}{dt} &= k_1 r_1 X + k_2 r_2 X + k_3 r_3 X + k_8 r_4 X - \frac{F}{V} X \\ \frac{dG}{dt} &= -r_G X + \frac{F}{V}\left(G^{in} - G\right) \\ \frac{dN}{dt} &= -r_4 X + \frac{F}{V}\left(N^{in} - N\right) \\ \frac{dE}{dt} &= k_4 r_2 X - r_3 X - \frac{F}{V} E \\ \frac{dT}{dt} &= \frac{1}{2} r_{sT} X - \frac{1}{2} r_{mT} X - \frac{F}{V} T \\ \frac{dA}{dt} &= k_7 r_2 - r_4 - A\left(k_1 r_1 + k_2 r_2 + k_3 r_3 + k_8 r_4\right) \\ \frac{dV}{dt} &= F \end{aligned} \tag{2}$$

where X, G, N, E are volumetric concentrations (g/L) of, respectively, biomass, external glucose, ammonium and ethanol, A is the intracellular concentration of α-ketoglutarate (g/gX), F is the feed flow rate, V is the culture volume, G^{in} and N^{in} are, respectively, the glucose concentration and ammonium concentration in the feed, and T (g/L) is the product between the intracellular trehalose concentration and biomass concentration. r_i, $i = 1 \dots 4$, are the reaction rates. The specific rate of respiration, the glucose uptake rate and ammonium uptake rate are given by

$$\begin{aligned} r_O &= \alpha \frac{K_I}{E + K_I} \\ r_G &= \mu_{Gmax} \frac{G}{G + K_G} \frac{K_{IA}}{(AX) + K_{IA}} \\ r_N &= \mu_{Nmax} \frac{N}{N + K_N} \frac{(AX)}{(AX) + K_A} \frac{K_{IA2}}{(AX) + K_{IA2}} \end{aligned} \tag{3}$$

while the overflow metabolism is expressed similarly as in (Sonnleitner and Käppeli, 1986) through the kinetic rates

$$\begin{aligned} r_1 &= \min\left(r_G, r_O\right) \\ r_2 &= \max\left(0, r_G - r_O\right) \\ r_3 &= \max\left(0, \left(r_O - r_G\right) \frac{E}{E + K_E}\right) \\ r_4 &= r_N \end{aligned} \tag{4}$$

The storage and mobilization of trehalose are described by the kinetic rates

$$r_{sT} = \mu_{sTmax} \cdot r_G\left(t-\tau_s\right) \cdot \frac{K_{IsN}}{N+K_{IsN}} \qquad (5)$$
$$r_{mT} = \mu_{mTmax} \cdot r_3\left(t-\tau_m\right) \cdot \frac{T}{T+K_{mT}}$$

which exhibit a delayed dependence (with time delay τ_s (h)) of trehalose storage on the extracellular glucose uptake rate r_G and a delayed dependence (with time delay τ_m (h)) of trehalose mobilization on ethanol respiration rate r_3. Additionally, the storage is inhibited by high ammonium concentration, while the mobilization is limited by trehalose availability.

The parameters of model (1)-(5) are identified from experimental data. The experimental database consists of four experiments, each of them lasting for 21 hours. The main difference between the experiments is given by the concentrations of nutrients glucose and ammonium in the influent flow. Two of these experiments consider ammonium starvation, meaning that no ammonium ($N^{in}=0$) is supplied in the feed flow from the starvation time onwards. Ammonium starvation has been proven to maximize intracellular accumulation of reserve carbohydrates such as trehalose (Wiemken, 1990). Measurements are collected for 15 to 16 time instants per experiment and include: biomass concentration, extracellular glucose concentration, ammonium concentration, ethanol concentration and intracellular trehalose concentration. The identification of the model without the dynamics of trehalose is described in (Richelle et al., 2014), while the identification of trehalose dynamics is detailed in (Huet et al., 2022). The model parameters employed in this study are given in Table 1.

Table 1. Model parameters

Name	Value	Units	Name	Value	Units
k_1	0.5998	gX/gG	μ_{Nmax}	1.1903	gN/gX/h
k_2	0.0662	gX/gG	K_G	0.1524	gG/L
k_3	0.9386	gX/gE	K_I	3.1817	gE/L
k_4	0.2452	gE/gG	K_E	0.1	gE/L
k_7	0.2389	gA/gX/gG	K_N	2.9370	gN/L
k_8	1.0150	gX/gN	K_A	9.0014	gA/L
α	0.4445	gG/gX/h	K_{IA}	5.5981	gA/L
μ_{Gmax}	2.5364	gG/gX/h	K_{IA2}	5.7737	gA/L
μ_{sTmax}	0.0500	gT/gG	K_{mT}	0.01	gT/L
μ_{mTmax}	0.4796	gT/gE	τ_s	6.080	h
K_{IsN}	0.3317	gN/L	τ_m	3.3905	h

4. MODEL-BASED OPTIMIZATION

4.1 Control variables and vector parameterization

The culture medium feeding is operated with a single pump. It contains glucose and ammonium concentrations respectively equal to $G^{in}=300$ g/L and $N^{in}=33$ g/L. To apply a nitrogen starvation, the culture medium can be replaced at a given time t_{Ns} with the same one except that $N^{in}=0$. To approximate the continuous time feed flow rate $F(t)$, a vector parameterization is used:

$$F(t)=F(k), \quad t_{k-1}\le t<t_k, \quad k=1\dots N \qquad (6)$$

where N is the number of constant feeding flow rates used for defining the feeding time profile and $F(k)$ are their corresponding values. A mesh refinement is used that consists in increasing the discretization in three successive steps, with $N=5$, $N=10$ and, finally, $N=20$. At each step, the total number of control variables is $N+1$, i.e., N constant plateaus $F(k)$ defining the feeding profile and the starvation time t_{Ns}.

4.2 Limit case studies: trehalose maximization or biomass maximization

We first consider the problem of maximizing the final intracellular trehalose concentration ($T(t_f)/X(t_f)$ in gT/gX), without any consideration on the final biomass amount. Hence, the optimization problem is defined as

$$\max_{F(k),\, t_{Ns}} J_T\left(F(k), t_{Ns}\right) = \max_{F(k),\, t_{Ns}} T(t_f)/X(t_f) \qquad (7)$$

subject to

$$\begin{aligned} &\text{system dynamics (2)-(5)} \\ &\max_t V(t) \le 9.5\,\text{L},\ 0\le t\le 21\,\text{h} \\ &0\le F(k)\le 0.9\,\text{L/h},\ k=1\dots N \\ &0\le t_{Ns}\le 21\,\text{h} \end{aligned} \qquad (8)$$

Note that in all the optimization problems considered in this paper, the following operating conditions have systematically been used (corresponding to the real Experiment 4 in the paper by Huet et al. (2022)):

- the duration of the culture is fixed: $t_f=21$ h;
- the initial conditions are fixed: $X(0)=0.13$ g/L, $G(0)=0.192$ g/L, $N(0)=0.29$ g/L, $E(0)=0.084$ g/L, $T(0)=0.005$ g/L, $A(0)=0$ gA/gX, $V(0)=6.5$ L;
- a sampling volume of 0.05 L is withdrawn from the culture every two hours from $t=0$ h to $t=12$ h, then every hour from $t=12$ h to $t=21$ h.

All the optimization problems were solved with the Matlab function `fminsearchcon` (with the tuning parameters `MaxIter` and `MaxFunEvals` set to $20*(N+1)$, $N+1$ being the total number of control variables). Initializing with a constant feeding at 0.17 L/h, i.e., almost the maximum constant feeding, and no nitrogen starvation,

$$F(k)=0.17\,\text{L/h},\ k=1\dots 5,\ t_{Ns}=t_f=21\,\text{h}, \qquad (9)$$

and solving the optimization problem (7) with constraints (8) leads to a final intracellular trehalose concentration $T(t_f)/X(t_f)=0.26$ gT/gX, a final biomass $X(t_f)V(t_f)=98$ g and a final biomass concentration $X(t_f)=10.9$ g/L. The time profiles of all the state variables are presented in Fig. 1. Nitrogen starvation is applied during the whole culture ($t_{Ns}=0$) and the culture medium is rapidly fed at the culture start. The final intracellular trehalose concentration is large in comparison with values found in the literature (e.g., 0.13 gT/gX in (Aranda et al., 2004), however, this result was obtained without process optimization). Values in that range are outside the validation range of the model (Huet et al., 2022). Maximizing the final intracellular trehalose leads, however, to a low final biomass amount.

At the opposite, we may consider the problem of maximizing the final biomass ($X(t_f)V(t_f)$ in g), without any consideration on the final intracellular trehalose concentration. Hence, the optimization problem is defined as

$$\max_{F(k),\, t_{Ns}} J_X\left(F(k), t_{Ns}\right) = \max_{F(k),\, t_{Ns}} X(t_f)V(t_f) \qquad (10)$$

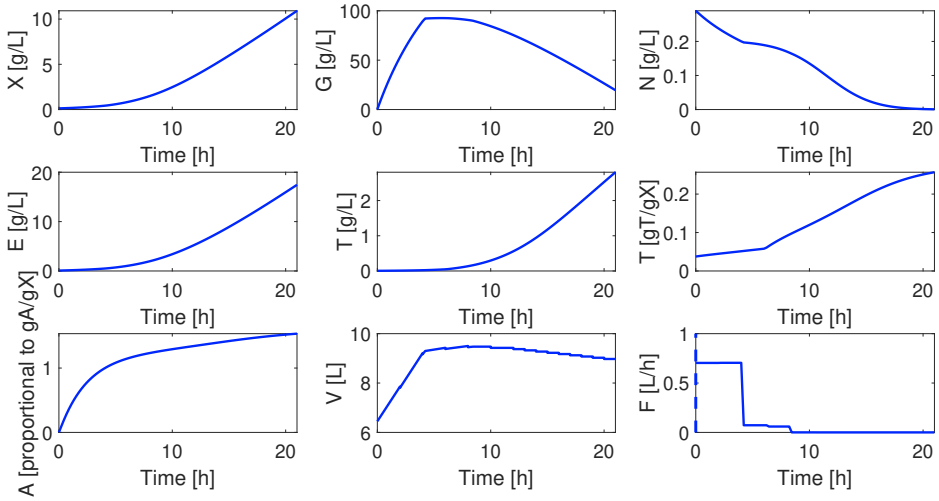

Fig. 1. State variables and optimal feeding (lower right) time profiles for trehalose maximization (solution of (7), (8)). Vertical blue dashed line indicates nitrogen starvation time.

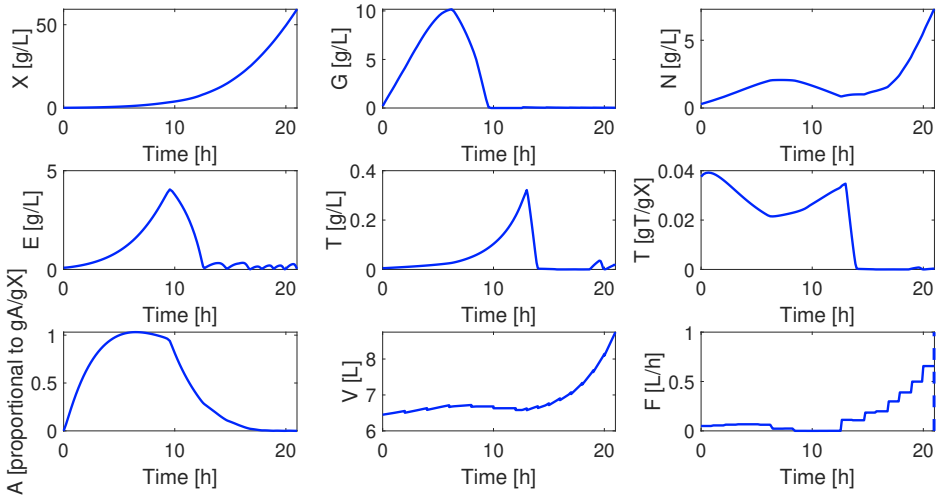

Fig. 2. State variables and optimal feeding (lower right) time profiles for biomass maximization (solution of (10), (8)). Vertical blue dashed line indicates nitrogen starvation time.

subject to (8). Initializing with a constant feeding at 0.01 L/h and no nitrogen starvation,

$$F(k) = 0.01\,\mathrm{L/h},\ k = 1\ldots 5,\ t_{Ns} = t_f = 21\,\mathrm{h}, \qquad (11)$$

and solving the optimization problem (10) with constraints (8) leads to a final intracellular trehalose concentration $T(t_f)/X(t_f) \approx 0$, a final biomass $X(t_f)V(t_f) = 519$ g and a final biomass concentration $X(t_f) = 59.3$ g/L. The time profiles of all the state variables are presented in Fig. 2. On the contrary to the case of trehalose maximization, nitrogen starvation is applied during the whole culture ($t_{Ns} = 21$ h). The feeding flow rate keeps at a low level during the first half of the culture, with a limited fermentation phase that accumulates ethanol up to 4 g/L. This ethanol is then consumed through respiration, followed by a respiration phase on glucose that is maintained with an exponential feeding flow rate, hence maximizing the yield from glucose to biomass. The final intracellular concentration is close to 0. These two limit cases show that maximizing trehalose and biomass are competing objectives. The next section tries to solve the problem with a multi-objective optimization of both trehalose and biomass.

4.3 Multi-objective optimization of trehalose and biomass

In order to simultaneously account for trehalose and biomass maximization, a multi-objective optimization problem is proposed:

$$\max_{F(k),\,t_{Ns}} J_{multi}\left(F(k), t_{Ns}\right) = \qquad (12)$$
$$\max_{F(k),\,t_{Ns}} \lambda J_T\left(F(k), t_{Ns}\right) w_T + (1-\lambda) J_X\left(F(k), t_{Ns}\right) w_X$$

subject to (8). $J_T(F(k), t_{Ns})$ and $J_X(F(k), t_{Ns})$ are, respectively, defined in (7) and (10), and $w_T = 1/0.25$, $w_X = 1/500$ are normalizing factors whose values are directly deduced from the optimal values obtained for J_T and J_X in section 4.2. The weight $\lambda \in [0, 1]$ drives the multi-objective cost function (12) between the two limit cases J_T (for $\lambda = 1$) and J_X (for $\lambda = 0$).

The solutions of (12), (8), starting from initial conditions (9) or (11), have been computed for several values of $\lambda \in [0, 1]$ (details not shown). For $\lambda \in [0.8, 1]$, the

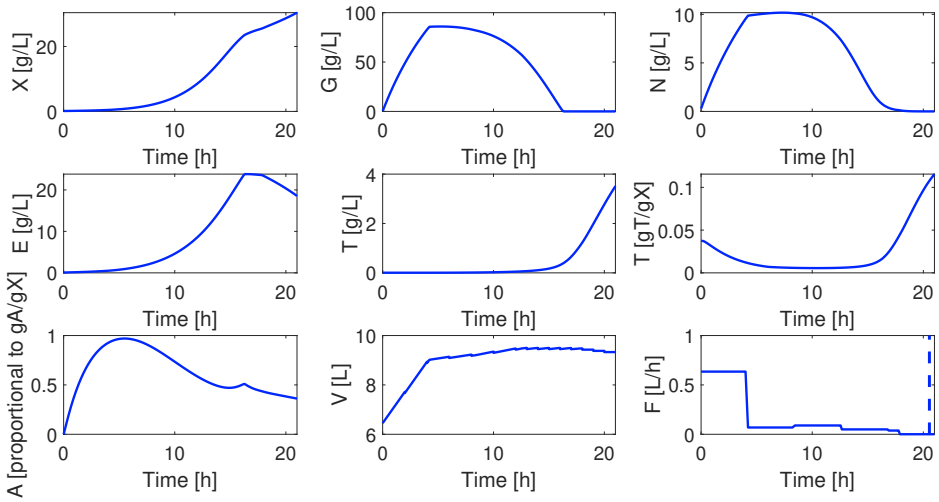

Fig. 3. State variables and optimal feeding (lower right) time profiles for multi-objective optimization (solution of (12), (8) with $\lambda = 0.5$). Vertical blue dashed line indicates nitrogen starvation time.

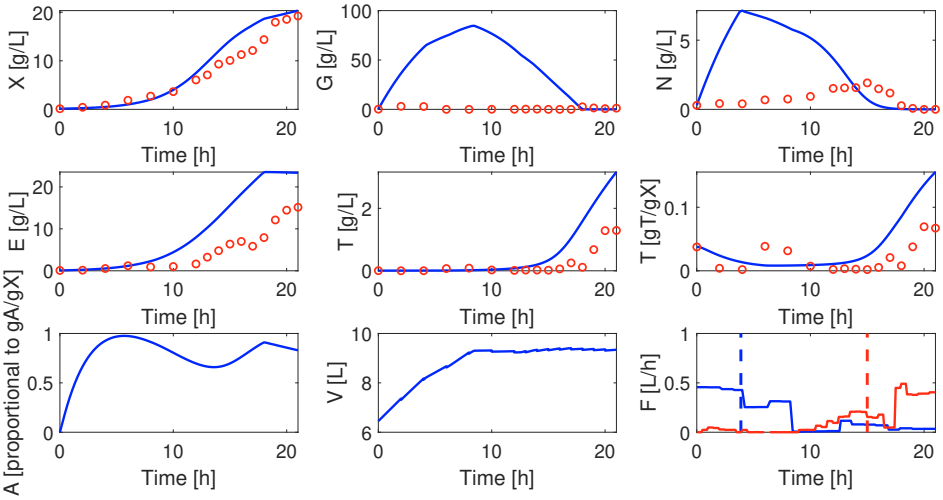

Fig. 4. State variables and optimal feeding (lower right) time profiles for constrained optimization (blue curves: solution of (7), (8), (13) with $X_{MIN} = 20$ g/L) compared to experimental results of Experiment 4 in Huet et al. (2022) (red circles and red curve). Vertical blue and red dashed lines indicate nitrogen starvation times. This figure shows that different feeding strategies can lead to similar biomass productions, but with very different trehalose accumulations.

results correspond to the solution obtained when maximizing trehalose, i.e., for $\lambda = 1$, presented in section 4.2 and Fig. 1. For $\lambda \in [0, 0.25]$, the results correspond to the solution obtained when maximizing biomass, i.e., for $\lambda = 0$, presented in section 4.2 and Fig. 2. For $\lambda \in [0.3, 0.75]$, the results systematically correspond to an intermediate solution, leading to a final intracellular trehalose concentration $T(t_f)/X(t_f) \approx 0.11$ gT/gX, a final biomass $X(t_f)V(t_f) \approx 280$ g and a final biomass concentration $X(t_f) \approx 30$ g/L. This result is a trade-off between trehalose maximization and biomass maximization. The results presented in Fig. 3 correspond to the optimal solution when solving (12), (8) with $\lambda = 0.5$ from initialization (11). There is a very short nitrogen starvation ($t_{Ns} = 20.5$ h), which is close to the limit case of biomass optimization, and most of the culture medium is fed during the first quarter of the culture, which is similar to the limit case of trehalose optimization. As we do not obtain a continuum of intermediate solutions, with different levels of trehalose accumulation and of biomass production, we propose in the next section another optimization problem to be solved, i.e., trehalose maximization constrained with a lower bound for the final biomass.

4.4 Trehalose maximization constrained with a lower bound for the final biomass

Aiming at maximizing final intracellular trehalose accumulation while preserving a lower bound on the final biomass amount, we define a new constrained optimization problem, i.e., (7), (8) to which an additional constraint is imposed:

$$X_{MIN}V_{MAX} \leq X(t_f)V(t_f) \tag{13}$$

where $V_{MAX} = 9.5$ L, according to (8), and X_{MIN} can be chosen *a priori*. We will consider $X_{MIN} \in [10, 30]$ g/L given, on the one hand, the biomass concentration obtained in section 4.2 when maximizing the final trehalose concentration ($X(t_f) = 10.9$ g/L) and, on the other hand, that we are looking for higher final trehalose concentrations than the one obtained in the intermediate result of section 4.3, i.e., $T(t_f)/X(t_f) \approx 0.11$ gT/gX with a

final biomass concentration $X(t_f) \approx 30$ g/L. One difficulty when using the Matlab function `fminsearchcon` for solving (7), (8), (13) is that we have to provide an initial feeding flow rate $F(k)$, $k = 1 \dots 5$, that satisfies all the constraints, including the additional one given in (13). This cannot be obtained with a constant feeding as was done in (9) or (11). Hence, we define an initial decreasing time profile of the type

$$F(k) = (5-k)F^*,\ k = 1 \dots 5,\ F^* = 0.083\text{L/h} \quad (14)$$

that satisfies (8), (13) $\forall X_{MIN} \in [10, 30]$ g/L. The results obtained by solving (7), (8), (13) with the initialization (14) are given in Table 2. A progressive trend is observed between the limit values of X_{MIN}: when the lower bound on the biomass concentration X_{MIN} increases, the final intracellular trehalose concentration $T(t_f)/X(t_f)$ decreases and the starvation phase starts later (at t_{Ns}). In Fig. 4, the optimal results for $X_{MIN} = 20$ g/L are compared with the experimental results obtained in the Experiment 4 in Huet et al. (2022) (from a set of 4 experiments used for the parameter identification of model (2)-(5)). Although the final biomass concentration is very similar (about 20 g/L), the other state variables exhibit significantly different time profiles given the strong differences between the optimal and the experimental feeding profiles. Feeding the culture medium essentially in the first half of the culture and starting the nitrogen starvation phase much earlier (after 4h instead of 15h), should help more than doubling the final accumulation of intracellular trehalose (0.16 gT/gX instead of 0.07 gT/gX).

Table 2. Optimal results obtained by solving (7), (8), (13) with the initialization (14)

X_{MIN} g/L	$T(t_f)/X(t_f)$ gT/gX	$X(t_f)V(t_f)$ g	$X(t_f)$ g/L	t_{Ns} h
10	0.26	98	11	0
15	0.17	143	16	1.6
20	0.16	190	20	3.9
25	0.14	238	25	10.5
30	0.11	285	31	18.1

5. CONCLUSION

In this paper, the two competing objectives of final intracellular trehalose maximization and final biomass maximization in *Saccharomyces cerevisiae* fed-batch cultures are analyzed with different approaches: independently, in a multi-objective optimization problem and, finally, in a constrained optimization problem where trehalose is maximized and a lower bound on the final biomass is included in the constraints. The multi-objective optimization can be driven with the weights on the two criteria, leading to three different solutions: the two limit cases and an intermediate one where the trehalose accumulation is limited to 0.11 gT/gX. More trade-off solutions can be obtained with the constrained optimization, e.g. a final trehalose accumulation of 0.16 gT/gX with a final biomass of 20 g/L, which is more than the double we obtained experimentally for the trehalose (0.07 gT/gX) with a suboptimal feeding profile. These optimal results should of course be validated experimentally in future research. Constraints on the ethanol accumulation could also be included in the optimization problem, depending on the application context, e.g. (almost) no ethanol production in biopharmaceutical applications or ethanol accumulation in baker's yeast production. Finally, closed-loop optimal control could be analyzed, using a state observer for the on-line estimation of the intracellular trehalose concentration.

REFERENCES

Aranda, J., Salgado, E., and Taillandier, P. (2004). Trehalose accumulation in *Saccharomyces cerevisiae* cells: experimental data and structured modeling. *Biochem. Eng. J.*, 17, 129–140.

Atasoy, I., Yuceer, M., and Berber, R. (2013). Optimization of operating conditions in fed-batch baker's yeast fermentation. *Chem. Process Eng.*, 34, 175–186.

Berber, R., Pertev, C., and Türker, M. (1998). Optimization of feeding profile for baker's yeast production by dynamic programming. *Bioprocess Eng.*, 20, 263–269.

Huet, A., Sbarciog, M., and Bogaerts, Ph. (2022). Macroscopic modeling of intracellular trehalose concentration in *Saccharomyces cerevisiae* fed-batch cultures. In *Proceedings of MATHMOD 2022*. Vienna.

Mahmoodi, M. and Nassireslami, E. (2021). Control algorithms and strategies of feeding for fed-batch fermentation of *Escherichia coli*: a review of 40 years of experience. *Prep. Biochem. Biotech.* doi: 10.1080/10826068.2021.1998112.

Ohtake, S. and Wang, Y. (2011). Trehalose: current use and future applications. *J. Pharm. Sci.*, 101, 2020–2053.

Picó, J., Garelli, F., Battista, H.D., and Mantz, R. (2009). Geometric invariance and reference conditioning ideas for control of overflow metabolism. *J. Process Contr.*, 19, 1617–1626.

Renard, F., Vande Wouwer, A., Valentinotti, S., and Dumur, D. (2006). A practical robust control scheme for yeast fed-batch cultures - an experimental validation. *J. Process Contr.*, 16, 855–864.

Richelle, A. and Bogaerts, Ph. (2014). Off-line optimization of baker's yeast production process. *Chem. Eng. Sci.*, 119, 40–52.

Richelle, A., Fickers, P., and Bogaerts, Ph. (2014). Macroscopic modelling of baker's yeast production in fed-batch cultures and its link with trehalose production. *Comput. Chem. Eng.*, 61, 220–233.

Saini, P., Beniwal, A., Kokkiligadda, A., and Vij, S. (2018). Response and tolerance of yeast to changing environmental stress during ethanol fermentation. *Process. Biochem.*, 72, 1–12.

Sonnleitner, B. and Käppeli, O. (1986). Growth of *Saccharomyces cerevisiae* is controlled by its limited capacity: formulation and verification of a hypothesis. *Biotechnol. Bioeng.*, 28, 927–937.

Tapia, H. and Koshland, D. (2014). Trehalose is a versatile and long-lived chaperone for desiccation tolerance. *Curr. Biol.*, 24, 2758–2766.

Valentinotti, S., Srinivasan, B., Holmberg, U., Bonvin, D., Cannizzaro, C., Rhiel, M., and von Stockar, U. (2003). Optimal operation of fed-batch fermentations via adaptive control of overflow metabolite. *Contr. Eng. Pract.*, 11, 665–674.

Wiemken, A. (1990). Trehalose in yeast, stress protectant rather than reserve carbohydrate. *Anton. Leeuw.*, 58, 209–217.

Yüzgeç, U., Türker, M., and Hocalar, A. (2009). Online evolutionary optimization of an industrial fed-batch yeast fermentation process. *ISA T.*, 48, 79–92.