



**HAL**  
open science

# An Experimental Assessment of Robust control and Estimation of Acetate Concentration in Escherichia coli BL21(DE3) Fed-batch Cultures

Merouane Abadli, Laurent Dewasme, Sihem Tebbani, Didier Dumur, Alain Vande Wouwer

► **To cite this version:**

Merouane Abadli, Laurent Dewasme, Sihem Tebbani, Didier Dumur, Alain Vande Wouwer. An Experimental Assessment of Robust control and Estimation of Acetate Concentration in Escherichia coli BL21(DE3) Fed-batch Cultures. Biochemical Engineering Journal, 2021, pp.108103. 10.1016/j.bej.2021.108103 . hal-03263524

**HAL Id: hal-03263524**

**<https://hal.science/hal-03263524v1>**

Submitted on 2 Aug 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

# An Experimental Assessment of Robust control and Estimation of Acetate Concentration in *Escherichia coli* BL21(DE3) Fed-batch Cultures

Merouane Abadli<sup>a,b</sup>, Laurent Dewasme<sup>b</sup>, Sihem Tebbani<sup>a</sup>, Didier Dumur<sup>a</sup>, Alain Vande Wouwer<sup>b</sup>

<sup>a</sup>Université Paris-Saclay, CNRS, CentraleSupélec, Laboratoire des Signaux et Systèmes, 3 rue Joliot-Curie, 91192 Gif sur Yvette, France

<sup>b</sup>Systems, Estimation, Control and Optimization (SECO), University of Mons, 31, Boulevard Dolez, 7000 Mons, Belgium;

---

## Abstract

Avoiding acetate accumulation is a major challenge in *Escherichia coli* fed-batch cultures, since it leads to an inhibition of the cell respiratory capacity. A closed-loop regulation ensuring a low acetate concentration offers a practical solution to maintain the cultures near the optimal operating conditions.

In this work, a robust Generic Model Controller (GMC) is designed and implemented to regulate the acetate concentration in *E. coli* BL21 (DE3) fed-batch cultures. To compensate for model mismatch, disturbances, and measurement noise, a robust design using the  $\mathcal{LMI}$  formalism is achieved, considering robustness and transient performance requirements. Since the acetate concentration is not available for on-line measurement, an Unscented Kalman Filter (UKF) is also designed and implemented to estimate the acetate concentration based on an on-line biomass measurement.

The proposed GMC-UKF strategy is validated through simulation runs and experimental tests at lab-scale. The control strategy shows good performance in regulating the non-measured acetate concentration. The estimation of this latter signal from the biomass measurement is performed accurately by the UKF.

*Keywords:* Feedback Linearization, Process Control, Kalman Filter, Biotechnology

---

## 1. Introduction

Recombinant proteins are widely used to produce a vast array of biopharmaceutical products. One of the common ways to achieve industrial-scale production is through fed-batch cultivation of genetically modified strains of *Escherichia coli*. The operational advantage is the scalable process, the low operational costs, and the relatively simple media conditions. On the other hand, *Escherichia coli* is known for its physiological and biological features, such as flexible culture conditions, fast growth and high production yields [1, 2].

The main challenge to ensure the process efficiency and productivity is the accumulation of acetate, a metabolic by-product inhibiting cell growth [3]. Acetate formation occurs when the capacity for energy generation within the cell is exceeded, due to high flux into the main metabolic pathways caused by an excess in the carbon source [4, 5]. This mechanism is referred to as overflow metabolism [6].

Acetate presence in high concentration causes the inhibition of the cell respiratory capacity, leading to the decrease of biomass production yield and consequently the decrease of the recombinant protein production [7, 8].

Keeping the feeding at a sufficiently low rate prevents acetate formation, but can lead to low productivity and a long cultivation time. It is therefore required to determine a feeding profile that maximizes biomass productivity while avoiding overflow metabolism [9].

Several optimization strategies and process control architectures have been developed to reduce or avoid overflow metabolism [10–17]. Basically, two different approaches can be followed. The first approach consists in controlling the specific biomass growth rate [18, 19] and to impose a reference biomass evolution profile. This type of control is made possible by the availability of reliable on-line biomass probes which allow convenient real-time implementation. However, the definition of a biomass reference profile is not straightforward as it relies on prior process knowledge (i.e., a growth model based on past experimental observations), and in practice, a suboptimal solution is often selected such as to ensure sufficient margin of security. Limiting the specific growth rate presents some practical and metabolic limitations, since its maximal level depends on the oxidative capacity of the cells which is by essence, uncertain. Therefore, targeting a growth rate close to its maximal value could lead to several uncontrolled metabolic switches provoking latencies. An example can be found in [20] where the glucose and oxygen consumption rates and CO<sub>2</sub> evolution rate suddenly and reproducibly decreased, causing a break of the metabolism for a period of 40 min, and a drop in the biomass productivity.

The second approach consists in regulating, either the substrate or the by-product concentration at specific levels [14–16]. The substrate concentration should be close to a threshold corresponding to the critical oxidation capacity, while the by-product concentration should be close to zero. The main obstacle is the difficulty of on-line implementation, due to the requirement of accurate measurements of low-level concentrations

41 of acetate and/or glucose.

42 It is reported in [21] that industrially relevant inhibitory levels of acetate concentration  
43 are on the order of 100 mM (6 g/L). The authors studied the effect of acetate presence in  
44 the culture medium on *E. coli* metabolism and showed that a concentration of 16.67 mM  
45 (1 g/L) corresponds to less than 20% drop in the growth rate compared to its maximal  
46 value.

47 In this context, the contribution of this paper is twofold:

- 48 • to propose a control strategy combining robust linearizing control with a software  
49 sensor to monitor and regulate the acetate concentration on-line. In this way, the  
50 need of an accurate process model is less pressing, and acetate concentration can  
51 be reconstructed from the biomass signal.
- 52 • to develop a proof of concept through experimental runs at lab-scale. Indeed, even  
53 though several control paradigms have been proposed in earlier publications there  
54 is a dire lack of reports on experimental validation, most of the published results  
55 being based on simulation studies.

56 To the authors' knowledge, the only successful report of an experimental application  
57 of linearizing control to fed-batch cultures of *Escherichia coli* cultures is published in  
58 [10], where the acetate concentration is regulated to a pre-defined set-point. However,  
59 the control strategy relies on an accurate knowledge of the model parameters, which is  
60 a major drawback since a bioprocess model is always uncertain. Parameter adaptation  
61 strategies are usually applied to compensate the uncertainty in the kinetic terms of the  
62 process model. However, stability is not guaranteed in presence of unmodeled dynamics  
63 and high noise levels, and this is why we propose a robust  $\mathcal{LMI}$ -based linearizing control,  
64 which will be able to alleviate this difficulty. Moreover, the control loop developed in [10]  
65 is based on a flux injection analysis (FIA) device, whose market distribution has long been  
66 disrupted, and no other similar device has been (re)developed in the meantime. The use  
67 of state estimation, or software sensors, seems therefore the most appropriate solution  
68 to avoid the burden of complex, unreliable, sensing techniques. The results reported in  
69 [10] show that exponential growth could not be sustained in the experimental studies,  
70 which might be an indication of the lack of accuracy of the FIA device. In this study, we  
71 propose an Unscented Kalman Filter (UKF) for the online reconstruction of the acetate  
72 concentration.

73 More specifically, we aim at the development of Generic Model Control (GMC) [22],  
74 which is an adaptive control strategy based on feedback linearization, embedding the  
75 process non-linearities in the design of the control law. GMC has been used in several  
76 process control applications, e.g. [23] where it was applied to track the foreign protein  
77 level reference trajectory in *E. coli* fermentations, [24] where it was applied to anaerobic  
78 digestion, and [25] where dual product composition was controlled in an industrial high  
79 purity distillation column. In this study, a robust version of the Generic Model Control

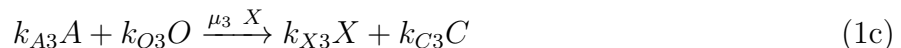
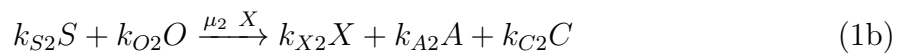
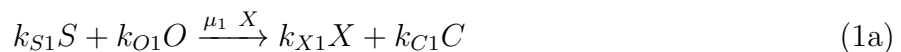
80 (GMC) strategy is developed to control the acetate concentration to a low predefined  
 81 value.  $\mathcal{LMI}$ s are considered in the control synthesis to derive the GMC control gains.  
 82 The control design includes performance requirements using the regional pole placement  
 83 technique. The approach ensures both the robust stability of the process in presence of  
 84 model uncertainties and measurement noise, and the desired transient performance of the  
 85 closed-loop system.

86 This paper is organized as follows. In section 2, the mechanistic model describing *E.*  
 87 *coli* growth is presented. The Generic Model Control strategy is presented and applied to  
 88 the *E. coli* model in section 3. In section 4, the robust control design approach with the  
 89  $\mathcal{LMI}$ s formulation is presented. Process observability analysis and acetate estimation  
 90 using an Unscented Kalman Filter (UKF) are developed in section 5. Numerical simula-  
 91 tion results are illustrated in section 6. Section 7 presents the materials and methods and  
 92 experimental results are detailed and discussed in section 8. Conclusions and perspectives  
 93 end this study in section 9.

## 94 2. Process dynamic model

95 In this section, we consider a generic mechanistic model describing *E. coli* growth in  
 96 fed-batch cultures.

97 This model describes *E. coli* cells catabolism through the following three main reac-  
 98 tions [26]:



99 where  $X, S, A, O, C$  are respectively, the concentrations in the culture medium of biomass,  
 100 substrate (glucose), acetate, dissolved oxygen, and carbon dioxide.  $k_{\xi i}$  ( $\xi = X, S, A, O, C$  ;  
 101  $i = 1, 2, 3$ ) are the yield coefficients.  $\mu_1, \mu_2$ , and  $\mu_3$  are the specific rates related to the  
 102 catabolic reactions describing substrate oxidation (1a), acetate production (fermentation)  
 103 (1b), and acetate oxidation (1c) [27]. Their proposed kinetic structures read: [28]:

$$\mu_1 = \frac{\min(q_s, q_{s_{crit}})}{k_{S1}} \quad (2a)$$

$$\mu_2 = \frac{\max(0, q_s - q_{s_{crit}})}{k_{S2}} \quad (2b)$$

$$\mu_3 = \frac{\min(0, q_{AC})}{k_{A3}} \quad (2c)$$

The kinetic terms related to consumption rates  $q_*$  are defined by:

$$q_s = q_{s_{max}} \frac{S}{K_s + S} \quad (3a)$$

$$q_{s_{crit}} = \frac{q_{O_{max}}}{k_{OS}} \frac{K_{iA}}{K_{iA} + A} \quad (3b)$$

$$q_{AC} = \frac{k_{OS}(q_{s_{crit}} - q_s)}{k_{OA}} \frac{A}{K_A + A} \quad (3c)$$

105 where  $q_s$  and  $q_{AC}$  denote the substrate and acetate consumption rates respectively,  
 106  $q_{s_{max}}$  and  $q_{O_{max}}$  are the maximal consumption rates for substrate and dissolved oxygen  
 107 respectively, and  $q_{s_{crit}}$  represents the substrate critical consumption rate. In the following,  
 108 mass balances are normalized with respect to the substrate and acetate ( $k_{S1} = k_{S2} = k_{A3}$   
 109 = 1).

110 The kinetic model (2) is based on the Sonnleitner and Käppeli bottleneck assumption  
 111 [29], applied to *Saccharomyces cerevisiae* (Figure 1). Two different operating modes can  
 112 be distinguished depending on the substrate concentration level. If the latter is higher  
 113 than the critical threshold corresponding to the available oxidative capacity ( $S > S_{crit}$ ),  
 114 acetate is produced by the cells through the fermentative metabolic pathway (reactions  
 115 (1a) and (1b)). The culture is said in respiro-fermentative mode (RF). Conversely, a  
 116 substrate concentration lower than the critical threshold ( $S < S_{crit}$ ) leads to substrate  
 117 and acetate (if present in the culture medium) oxidation (reactions (1a) and (1c)), and  
 118 the culture is said in respirative mode (R). When the substrate concentration is at the  
 119 critical level and fills exactly the respirative capacity, the culture is in optimal condi-  
 120 tions corresponding to the edge between the two operating modes, and acetate is neither  
 121 produced nor consumed.

Applying component-wise mass balances to (1), we obtain the following differential equations [28]:

$$\dot{X} = (k_{X1}\mu_1 + k_{X2}\mu_2 + k_{X3}\mu_3)X - D X \quad (4a)$$

$$\dot{S} = -(\mu_1 + \mu_2)X - D (S - S_{in}) \quad (4b)$$

$$\dot{A} = (k_{A2}\mu_2 - \mu_3)X - D A \quad (4c)$$

$$\dot{O} = -(k_{O1}\mu_1 + k_{O2}\mu_2 + k_{O3}\mu_3)X - D O + OTR \quad (4d)$$

$$\dot{C} = (k_{C1}\mu_1 + k_{C2}\mu_2 + k_{C3}\mu_3)X - D C - CTR \quad (4e)$$

$$\dot{V} = F_{in} \quad (4f)$$

122 where  $S_{in}$  is the substrate concentration in the feed, and  $F_{in}$  is the inlet feed rate.  $V$  is  
 123 the culture medium volume and  $D$  is the dilution rate ( $D = \frac{F_{in}}{V}$ ).

124  $OTR$  and  $CTR$  represent respectively the oxygen transfer rate from the gas phase to  
 125 the liquid phase and the carbon transfer rate from the liquid phase to the gas phase, that  
 126 can be modeled as follows [30]:

$$OTR = k_L a_O (O_{sat} - O) \quad (5)$$

$$CTR = k_L a_{CO_2} (C - C_{sat}) \quad (6)$$

127 where  $k_L a_O$  and  $k_L a_{CO_2}$  are respectively the volumetric transfer coefficients of oxygen and  
 128 carbon dioxide.  $O_{sat}$  and  $C_{sat}$  are respectively the dissolved oxygen and carbon dioxide  
 129 concentrations at saturation.

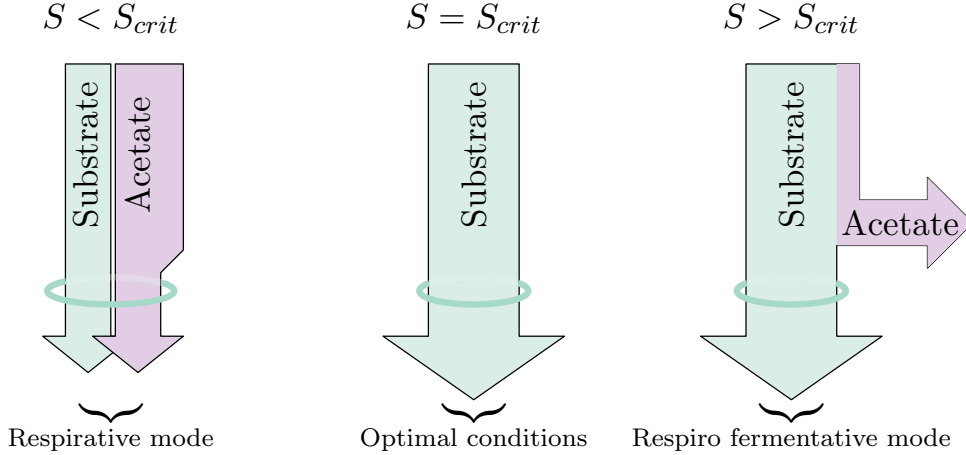


Figure 1: Illustration of the bottleneck assumption ([29]) describing the limited respiratory capacity

The optimal operating conditions maximizing the biomass productivity are at the boundary of the respiro-fermentative and respirative modes [14], where all the available substrate is assumed to be allocated for biomass production. Thus, the specific fermentation rate  $\mu_2$  and the specific acetate oxidation rate  $\mu_3$  are equal to zero:

$$\mu_1 = q_s = q_{s_{crit}} \quad (7)$$

$$\mu_2 = 0 \quad (8)$$

$$\mu_3 = 0 \quad (9)$$

130 Maintaining the culture at the edge between the respirative and respiro-fermentative  
 131 modes requires controlling the substrate concentration to the critical value  $S_{crit}$ . An  
 132 efficient on-line substrate measurement around this value is required [14], but the con-  
 133 centration level is below the resolution of currently available glucose probes.

134 At the optimal operating conditions, the acetate quantity ( $VA$ ) is constant. How-  
 135 ever, the volume evolution is exponential, and the acetate concentration must therefore  
 136 decrease with the same rate to maintain  $VA$  constant and reach the optimal operating  
 137 conditions. This is a difficult task, especially at a concentration lower than the sensitivity  
 138 level of the measurement and estimation tools.

139 A practical sub-optimal solution to these limitations is to control the acetate concen-  
 140 tration around a low value  $A_{ref}$ , depending on the sensitivity of the measurement devices  
 141 and the accuracy of the estimation methods.

### 142 3. Generic Model Control (GMC)

#### 143 3.1. GMC background

The Generic Model Control (GMC) is a two-step control approach developed by Lee and Sulivan [31]. In a first step, a feedback linearizing controller is derived (as illustrated in Figure 2) assuming a perfect process knowledge, and allowing the linearization of the nonlinear process behavior. In a second step, the output is forced to track a reference trajectory using a proportional-integral controller. Consider the following single-input single-output (SISO) system:

$$\frac{d}{dt}x = f(x) + g(x)u \quad (10)$$

$$y = h(x) \quad (11)$$

where  $x \in \mathbb{R}^n$  is the state vector,  $y \in \mathbb{R}^m$  is the process output vector,  $f$  and  $g$  are nonlinear functions, and  $h$  is the measurement function. The time derivative of the output  $y$  is given by:

$$\frac{d}{dt}y = \dot{y} = \frac{\partial h}{\partial x} \dot{x} = \frac{\partial h}{\partial x} [f(x) + g(x)u] = L_f h(x) + L_g h(x)u \quad (12)$$

where  $L_{\bullet}h(x) = \frac{\partial h}{\partial x} \bullet(x)$  is the Lie derivative of  $h$  along  $\bullet$ . If  $L_g h(x) \neq 0$  the following input:

$$u = \frac{1}{L_g h(x)} (-L_f h(x) + \hat{u}) \quad (13)$$

144 leads to a linear relation between  $y$  and the fictive input  $\hat{u}$  ( i.e.  $\dot{y} = \hat{u}$ ) [32]. The  
 145 equivalent linear model is then coupled to a proportional integral (PI) controller of the  
 146 form:

$$\hat{u} = \lambda_1(y_{ref}(t) - y(t)) + \lambda_2 \int_0^t (y_{ref}(\tau) - y(\tau))\partial\tau \quad (14)$$

147 where  $y_{ref}$  is the reference, and  $\lambda_1$  and  $\lambda_2$  are the controller parameters chosen accord-  
 148 ingly to the desired closed-loop system behavior. The integral action role is to account  
 149 for model uncertainties, nonlinearities, and disturbances.

150



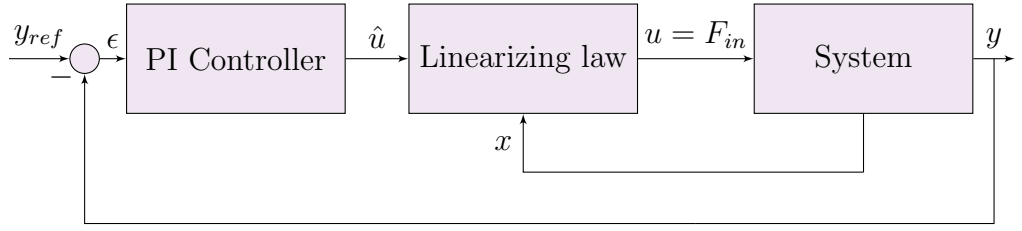


Figure 2: Generic model control scheme

151 The nonlinear closed-loop stability and the performance analysis of the GMC algo-  
 152 rithm are detailed in [33]. Robust stability is ensured for any positive values of  $\lambda_1$  and  $\lambda_2$ .  
 153 The proof is based on finding a strict Lyapunov function for the nominal process model  
 154 and on applying a perturbation theorem. Another stability proof for a similar control  
 155 structure with kinetic parameter estimation using the Kalman filter is given in [17].

### 156 3.2. Application of GMC to *E. coli* cultures

157 GMC is applied to an *E. coli* culture as illustrated in Figure 3. Considering acetate  
 158 concentration as the controlled output, and assuming its availability for measurement  
 159 ( $y = A$ ).

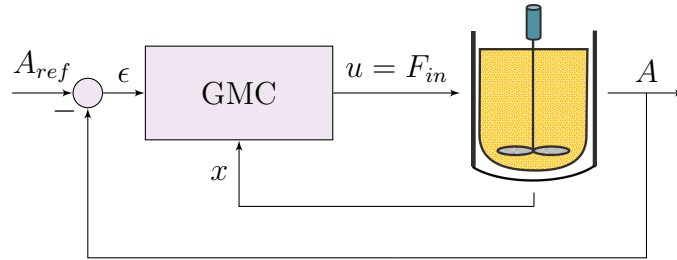


Figure 3: Generic model control applied to fed-batch *E. coli* cultures

160 As the theoretical value of  $S_{crit}$  is very small (below 0.1 g/L) and assuming a quasi-  
 161 steady state of  $S$  (i.e. no accumulation of glucose in the neighborhood of the optimal  
 162 operating conditions), the small quantity of substrate  $VS$  is almost instantaneously con-  
 163 sumed by the cells ( $\frac{d(VS)}{dt} \approx 0$  and  $S \approx 0$ ), and Equation (4b) yields:

$$\mu_2 X = -\mu_1 X + DS_{in} \quad (15)$$

164 where  $\mu_1$  and  $\mu_2$  are nonlinear functions of  $S$ ,  $A$  and  $O$  as given by equations (2) and (3).  
 165 Replacing  $\mu_2 X$  by Equation (15), the mass balance equation of  $A$  (Equation (4c)) can be  
 166 expressed as:

$$\dot{A} = -k_{A2}\mu_1 X - \mu_3 X - u(A - k_{A2}S_{in}) \quad (16)$$

where  $u = D = \frac{F_{in}}{V}$  is the control input. Applying the GMC scheme yields:

$$\dot{A} = \hat{u} = \lambda_1(A_{ref} - A) + \lambda_2 \int_0^t (A_{ref} - A) \partial\tau \quad (17)$$

Equating (16) and (17), the following control law is obtained:

$$F_{in} = V \frac{\hat{u} + (k_{A2}\mu_1 + \mu_3) X}{k_{A2}S_{in} - A} \quad (18)$$

$$\hat{u} = \lambda_1(A_{ref} - A) + \lambda_2 \int_0^t (A_{ref} - A) \partial\tau \quad (19)$$

167 where  $(k_{A2}\mu_1 + \mu_3)$  is an assumed uncertain kinetic term. The next section therefore  
 168 explores a robust control design in order to compensate this uncertainty.

#### 169 4. Robust control design

The linearizing control law obtained in the previous section can be written in the following form:

$$F_{in} = V \frac{\hat{u} + \theta X}{k_{A2}S_{in} - A} \quad (20)$$

$$\hat{u} = \lambda_1(A_{ref} - A) + \lambda_2 \int_0^t (A_{ref} - A) \partial\tau$$

where  $\theta$  is the kinetic term given by:

$$\theta = k_{A2}\mu_1 + \mu_3 \quad (21)$$

Structural and parametric uncertainties as well as estimation errors can be lumped into a global parametric error:

$$\delta = \bar{\theta} - \theta \quad (22)$$

where  $\delta$  is a nonlinear function of  $(S, A, O)$  representing possible inexact cancellations of nonlinear terms due to model uncertainties, and  $\bar{\theta}$  represents the hypothetical exact (unknown) value. Rewriting the control law in Equation (20) using the new expression of the kinetic term from Equation (22), we obtain:

$$F_{in} = V \frac{\hat{u} + \bar{\theta} X - \delta X}{k_{A2}S_{in} - A} \quad (23)$$

which corresponds to the perturbed reference system:

$$\dot{A} = \hat{u} - \delta X \quad (24)$$

Following a similar approach to the one developed in [14], the time-varying parameter  $\delta$  is assumed bounded and belonging to the set  $\Delta$  defined by:

$$\Delta := \{\delta : \underline{\delta} \leq \delta \leq \bar{\delta}\} \quad (25)$$

170 with  $\underline{\delta}$  and  $\bar{\delta}$  respectively representing the minimal and maximal values of the assumed  
171 bounded polytope set.

172 The control parameters  $\lambda_1$  and  $\lambda_2$  are designed to ensure some robustness and tracking  
173 performance to the overall closed-loop system. To this end, the acetate tracking error  
174 ( $\tilde{A}_1 = A_{ref} - A$ ) dynamics can be modeled by the following augmented system, illustrated  
175 in Figure 4:

$$\begin{aligned} \dot{\tilde{A}}_1 &= \frac{d}{dt}(A_{ref} - A) = -\hat{u} + \delta X \\ \dot{\tilde{A}}_2 &= A_{ref} - A = \tilde{A}_1 \end{aligned} \quad (26)$$

Considering the state vector  $x = \tilde{A} = [\tilde{A}_1 \ \tilde{A}_2]^T \in \mathbb{R}^n$ , the performance output  $e = \tilde{A}_1 = (A_{ref} - A) \in \mathbb{R}^{n_e}$  and the disturbance  $w = [X \ A_{ref}]^T \in \mathbb{R}^{n_w}$ , the control problem can be formulated as a state feedback controller ( $\hat{u} = Kx$ ) applied to the augmented system  $\mathcal{M}$ :

$$\mathcal{M} : \begin{cases} \dot{x} = A_{\mathcal{M}}x + B_w w + B_u \hat{u} \\ e = C_e x + D_{ew} w + D_{eu} \hat{u} \end{cases} \quad (27)$$

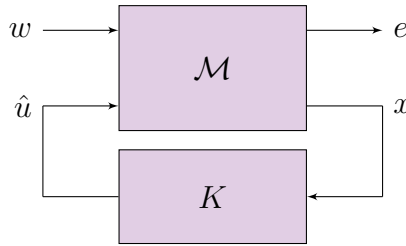


Figure 4: Robust control scheme

The state-space matrices are given by:

$$\begin{aligned} A_{\mathcal{M}} &= \begin{bmatrix} 0 & 0 \\ 1 & 0 \end{bmatrix} & B_w &= \begin{bmatrix} \delta & 0 \\ 0 & 0 \end{bmatrix} & B_u &= \begin{bmatrix} -1 \\ 0 \end{bmatrix} \\ C_e &= [1 \ 0] & D_{ew} &= [0 \ 0] & D_{eu} &= 0 \end{aligned} \quad (28)$$

and the representation of the closed-loop system is therefore given by:

$$\begin{bmatrix} \dot{x} \\ e \end{bmatrix} = \begin{bmatrix} A_f & B_f \\ C_f & D_f \end{bmatrix} \begin{bmatrix} x \\ w \end{bmatrix} = \begin{bmatrix} A_{\mathcal{M}} + B_u K & B_w \\ C_e + D_{eu} K & D_{ew} \end{bmatrix} \begin{bmatrix} x \\ w \end{bmatrix} \quad (29)$$

#### 176 4.1. Robustness constraints

177 The control design problem consists in determining the controller parameters  $\lambda_1$  and  
178  $\lambda_2$  so as to limit the infinity norm of the closed-loop transfer function within a predefined  
179 performance index, ( $\|\mathbf{T}(s) = D_f + C_f (s\mathbb{I}_n - A_f)^{-1} B_f\|_{\infty} < \gamma_{\infty}$ ), where  $s$  is the Laplace  
180 variable. First, the following assumptions on the plant parameters are considered:

181 **Assumption 1.** *The pair  $(A_{\mathcal{M}}, B_u)$  and  $(A_{\mathcal{M}}, C_e)$  are respectively stabilizable and de-*  
182 *tectable*

183 **Assumption 2.**  $D_{yu} = \mathbb{O}_{n_y, n_u}$

184 Under the previous assumptions, the Bounded Real Lemma [34] for continuous-time  
185 systems gives an equivalent  $\mathcal{LMI}$  formulation of the control problem:

**Lemma 1.** *The  $\mathcal{H}_{\infty}$  norm of the continuous-time transfer function  $\mathbf{T}(s)$  associated to the closed-loop system (29) is strictly smaller than  $\gamma_{\infty}$  if and only if there exists a symmetric positive definite matrix  $Q_{\infty}$  verifying:*

$$Q_{\infty} > 0 \quad \begin{bmatrix} A_f Q_{\infty} + Q_{\infty} A_f^T & B_f & Q_{\infty} C_f^T \\ B_f^T & -\gamma_{\infty} \mathbb{I}_{n_w} & D_f^T \\ C_f Q_{\infty} & D_f & -\gamma_{\infty} \mathbb{I}_{n_e} \end{bmatrix} < 0 \quad (30)$$

According to the bounded real lemma, the closed-loop system (29) is stable if and only if there exists:  $Q_{\infty} = Q_{\infty}^T > 0$  verifying:

$$\begin{bmatrix} A Q_{\infty} + B_u K Q_{\infty} + Q_{\infty} A^T + Q_{\infty} K^T B_u^T & B_w & Q_{\infty} C_e^T + Q_{\infty} K^T D_{eu}^T \\ B_w^T & -\gamma_{\infty} \mathbb{I}_{n_w} & D_{ew}^T \\ C_e Q_{\infty} + D_{eu} K Q_{\infty} & D_{ew} & -\gamma_{\infty} \mathbb{I}_{n_e} \end{bmatrix} < 0 \quad (31)$$

Considering  $L = K Q_{\infty}$ , the following  $\mathcal{LMI}$  is obtained:

$$\begin{bmatrix} A Q_{\infty} + B_u L + Q_{\infty} A^T + L^T B_u^T & B_w & Q_{\infty} C_e^T + L^T D_{eu}^T \\ B_u^T & -\gamma_{\infty} \mathbb{I}_{n_w} & D_{ew}^T \\ C_e Q_{\infty} + D_{eu} L & D_{ew} & -\gamma_{\infty} \mathbb{I}_{n_e} \end{bmatrix} < 0 \quad (32)$$

186 and the controller given by  $K = L Q_{\infty}^{-1}$  ensures a level of robustness w.r.t the bounded  
187 uncertainty  $\delta$ . Next, the desired performance constraints are defined and added to the  
188 robustness condition (32).

189 *4.2. Performance constraints*

190 Besides ensuring the robustness of the closed-loop, it is desirable to achieve some  
 191 performance in terms of the transient response (e.g., damping, response time, etc.). In  
 192 other words, constraints are added to the location of closed-loop poles of system (29).

193 For a second-order system with poles  $\lambda = -\zeta\omega_n \pm j\omega_d$ , the step response is character-  
 194 ized by the undamped natural frequency  $\omega_n = |\lambda|$ , the damping ratio  $\zeta$ , and the damped  
 195 natural frequency  $\omega_d$ . To ensure a desired transient response, specific bounds are imposed  
 196 on these quantities, thus constraining the closed-loop poles  $\lambda$  in a prescribed region of  
 197 the complex plane. Pole placement constraints can be expressed using  $\mathcal{LM}\mathcal{I}$  regions,  
 198 which are known to have interesting geometric properties for control purposes (convexity,  
 199 symmetry, ...) [34]. A suitable region satisfying this criterion is the intersection of the  
 200 half-plane  $s < -\alpha < 0$ , the disk of radius  $r$  and the conic sector defined by an angle  $\Theta$ .  
 201 The corresponding region  $\mathcal{S}(\alpha, r, \Theta)$  is defined as follows:

$$\mathcal{S}(\alpha, r, \Theta) = \{a < -\alpha < 0, \quad |s = a + jb| < r, \quad \tan \Theta a < -|b|\} \quad (33)$$

202 In this way, it is possible to set a minimum decay rate  $\alpha$ , a minimum damping ratio  
 203  $\zeta = \cos(\Theta)$ , and a maximum undamped natural frequency  $\omega_d = r \sin(\Theta)$  [35].

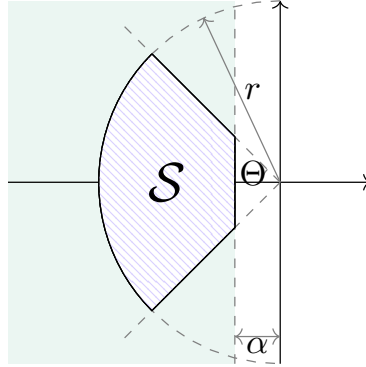


Figure 5: Representation of the region  $\mathcal{S}(\alpha, r, \Theta)$

204 The poles of the closed-loop system (29) are contained in the region  $\mathcal{S}(\alpha, r, \Theta)$ , if there  
 205 exists a symmetric positive definite matrix  $Q = Q^T$  verifying [34]:

$$\begin{aligned} & A_f Q + Q A_f^T + 2\alpha Q < 0 \\ & \begin{bmatrix} -rQ & A_f Q \\ Q A_f^T & -rQ \end{bmatrix} < 0 \\ & \begin{bmatrix} \sin \Theta (A_f Q + Q A_f^T) & \cos \Theta (A_f Q - Q A_f^T) \\ \cos \Theta (Q A_f^T - A_f Q) & \sin \Theta (A_f Q + Q A_f^T) \end{bmatrix} < 0 \end{aligned} \quad (34)$$

206 Our control design problem consists then in finding a state-feedback gain  $K$  that:

- 207 • guarantees the  $H_\infty$  performance  $\|\mathbf{T}(s)\|_\infty < \gamma_\infty$ .
- 208 • places the closed-loop poles in the  $\mathcal{LM}\mathcal{I}$  region  $\mathcal{S}(\alpha, r, \Theta)$  defined by Equation (33).

209 The first criterion (robustness) is ensured by solving Equation (32), and computing  
 210 the matrix  $Q_\infty$ . On the other hand, a sufficient condition to ensure the performance  
 211 constraints given by Equation (34) is to take  $Q = Q_\infty$ , yielding:

$$\begin{aligned}
 & A_f Q_\infty + Q_\infty A_f^T + 2\alpha Q_\infty < 0 \\
 & \begin{bmatrix} -rQ_\infty & A_f Q_\infty \\ Q_\infty A_f^T & -rQ_\infty \end{bmatrix} < 0 \\
 & \begin{bmatrix} \sin \Theta (A_f Q_\infty + Q_\infty A_f^T) & \cos \Theta (A_f Q_\infty - Q_\infty A_f^T) \\ \cos \Theta (Q_\infty A_f^T - A_f Q_\infty) & \sin \Theta (A_f Q_\infty + Q_\infty A_f^T) \end{bmatrix} < 0
 \end{aligned} \tag{35}$$

212 The robust GMC control design procedure based on  $\mathcal{LM}\mathcal{I}$ s is summarized in the  
 213 following steps:

- 214 • **Step1:** Select a suitable range for the uncertain variable  $\delta$ .
- 215 • **Step2:** Determine the values of  $\alpha$ ,  $r$ ,  $\Theta$  in order to meet a suitable transient  
 216 performance.
- 217 • **Step3:** Solve (off-line) the bounded real lemma (Equation (32)) and the perfor-  
 218 mance  $\mathcal{LM}\mathcal{I}$  (Equation (35)) simultaneously, to compute the gain  $K$ , and obtain  
 219 the robust GMC controller parameters  $\lambda_1$  and  $\lambda_2$ .

## 220 5. Acetate estimation

221 The acetate concentration needs to be determined on-line to apply the proposed GMC  
 222 strategy and drive the system close to the optimal operating conditions. However, no  
 223 device presenting a sufficient level of accuracy is currently available on the market. This  
 224 study proposes estimating the acetate and glucose concentrations from the viable biomass  
 225 concentration signal, which is efficiently measurable with low noise amplitudes via spec-  
 226 trophotometric probes. However, before implementing a state estimation algorithm, the  
 227 mechanistic model presented in section 2 has to be observable.

228 Observability is a system property that relates to the possibility of estimating the  
 229 system state based on the available measurement information. A detailed analysis of the  
 230 observability of the bioprocess model under consideration is given in [30]. The biomass  
 231 concentration measurement is sufficient to reconstruct the acetate concentration as long  
 232 as the substrate concentration remains at a sufficiently low level ( $0 \text{ g/L} < S < 0.1 \text{ g/L}$ ).

233 Since the bioprocess's observability is guaranteed, acetate concentration can be esti-  
 234 mated using a state observer, which can take various forms [36]. In [30], an extended

235 Kalman filter (EKF) was applied -under specific conditions- to estimate the glucose and  
 236 acetate concentrations using various sensor configurations. Whereas the EKF was able  
 237 to reconstruct the acetate concentration from the biomass measurement efficiently, the  
 238 strong nonlinearities in the kinetic model (4) can lead to significant estimation errors and  
 239 a low convergence rate EKF based on the linearization of the nonlinear process.

240 In the present study, the Unscented Kalman Filter (UKF) [37, 38] is considered.  
 241 Unlike the EKF, the UKF does not involve any linearization around the current state  
 242 estimate. A set of sample points is propagated through the nonlinear system, which allows  
 243 the reconstruction of the state estimate's mean and covariance under the assumption of  
 244 a Gaussian distribution of the noise.

245 The UKF algorithm implemented in this study is detailed in [39], where it was imple-  
 246 mented for a similar overflow mechanistic model for fed-batch cultures of hybridoma cells.  
 247 Since the biomass measurements are provided in discrete samples of time, the continuous-  
 248 discrete version of the UKF is implemented, i.e., a continuous-time model prediction and  
 249 a discrete-time measurement update.

250 The UKF estimator combined with the robust GMC controller applied to the *E. coli*  
 251 culture is illustrated in Figure 6.

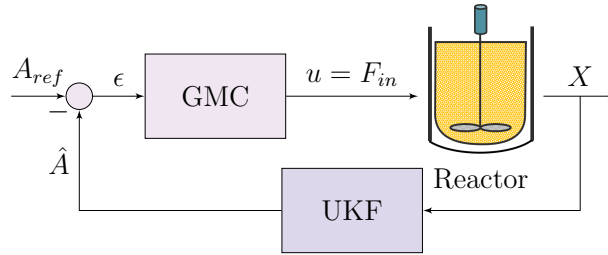


Figure 6: Generic Model Control combined with the Unscented Kalman filter applied to fed-batch *E. coli* cultures

## 252 6. Numerical simulations

253 In this section, several numerical simulations are achieved before the experimental  
 254 validation. First, the estimation of the acetate concentration using the Unscented Kalman  
 255 filter is tested. Second, the performance and robustness of GMC are assessed assuming  
 256 that the acetate concentration is available for measurement. Finally, the estimation and  
 257 control algorithms are combined. The cultures are achieved in a 5-L bioreactor and the  
 258 kinetic and stoichiometric parameters are those estimated in a previous work [28].

259 A predefined feeding profile is applied to the system, consisting of a batch phase  
 260 followed by a fed-batch phase with an exponential feeding profile. The UKF tuning  
 261 parameters  $(\alpha, \beta, \kappa)$ , the process and measurement noise covariance matrices  $Q$  and  $R$ ,  
 262 and the initial state covariance matrix  $P_0$  are given in Table 1.

Table 1: UKF covariance matrices, sigma point tuning parameters, and initial conditions

Parameter	Value	Unit
$Q$	$diag[10^{-4}, 10^{-2}, 10^{-2}, 10^{-8}]$	g/L
$R$	$10^{-4}$	g/L
$P_0$	$10^{-4} \times eye(N)$	g/L
$[\alpha, \beta, \kappa]$	$[1, 2, 0]$	-
$X_0$	0.1	g/L
$S_0$	5	g/L
$A_0$	0.1	g/L
$V_0$	3.5	L
$S_{in}$	500	g/L

263 The convergence of the UKF is first tested with (largely) erroneous initial conditions.  
 264 Figure 7 shows the performance of the UKF in estimating glucose and acetate concentra-  
 265 tions based on the biomass concentration measurement affected by additive white noise  
 266 with zero mean and a standard deviation of 0.1 g/L. After a transient phase of 3 h, both  
 267 states are well estimated and the convergence is achieved. The mean square errors of the  
 268 substrate and acetate estimates during this test are:  $\overline{e_S} = 0.53$  g/L and  $\overline{e_A} = 0.23$  g/L  
 269 respectively, which include the initial transient phase but are coherent with the sensitivity  
 270 of the measurements and the noise levels (0.1 g/L).

271 Further, the performance of the robust GMC design based on the  $\mathcal{LM}\mathcal{I}$  approach with  
 272 the regional pole assignment is tested. The control objective is to regulate the acetate set-  
 273 point  $A_{ref}$ , chosen sufficiently low to approach the neighborhood of the optimal trajectory  
 274 but also sufficiently high to stay within the limit of the observation sensitivity (0.1 g/L)  
 275 and maintain the culture in respiro-fermentative mode. The acetate concentration is  
 276 assumed available on-line for feedback, with the consideration of measurement noise.

The first step in our design approach is to define upper and lower bounds for the  
 parametric uncertainty  $\delta$ . The expression of the kinetic parameter  $\theta$  is given by:

$$\theta = k_{A2}\mu_1 + \mu_3 \quad (36)$$

277 The expression of the uncertain term  $\theta$ , and the kinetic terms  $\mu_1$  and  $\mu_3$  contain the pa-  
 278 rameters  $k_{A2}, K_s, K_{iA}, k_{OS}, K_A$ . These parameters can deviate from their nominal values,  
 279 thereby deviations of maximum 15% are considered. Consequently, the range  $\Delta$  can be  
 280 defined by  $\underline{\delta} = 0$  and  $\overline{\delta} = 0.1$ .

281 Regarding the performance constraints, we desire to enforce a maximal settling time  
 282  $T_s = \frac{4}{\zeta\omega_n} = \frac{4}{\alpha}$  equal to 2 h, and to prevent fast controller dynamics.

283 To this end, we characterize the section  $\mathcal{S}(\alpha, r, \theta)$  as the intersection of the half-plane  
 284  $x < -\alpha = -\frac{4}{T_s}$  with the disk of radius  $r = 4$  centred at the origin, and the conic section  
 285 defined by  $\Theta = \frac{\pi}{2}$  rad.

In light of these constraints, the  $\mathcal{LM}\mathcal{I}s$  (Equations (32) and (35)) are solved numer-



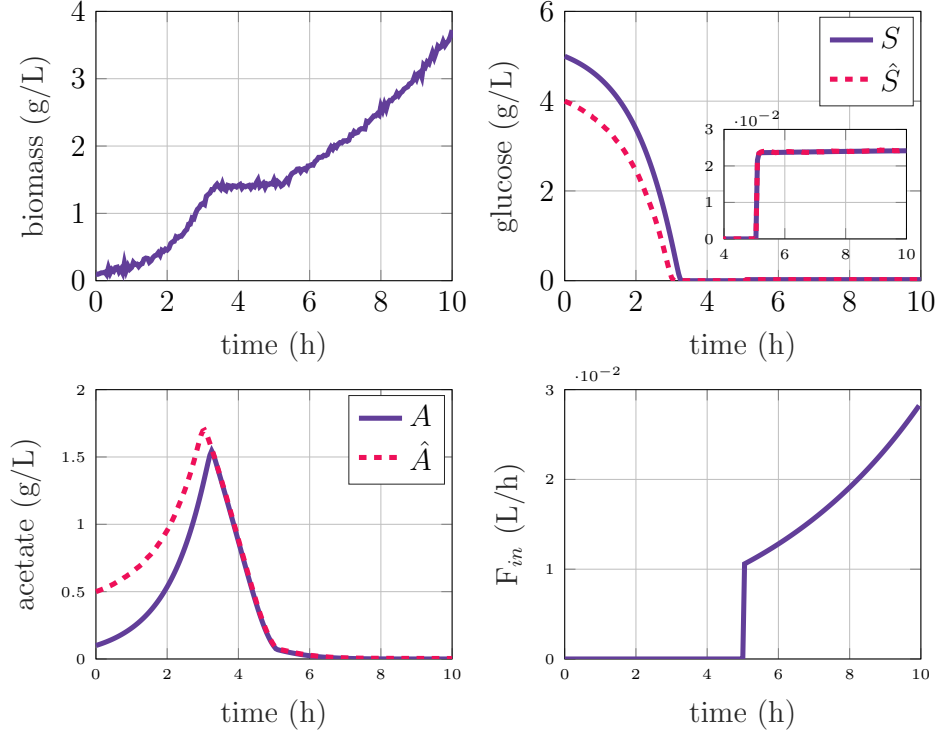


Figure 7: Convergence of UKF starting from erroneous initial conditions - estimation of glucose and acetate concentrations based on biomass concentration measurements.

ically using the solver *SeDuMi* [40] and the following results are obtained:

$$Q_\infty = \begin{bmatrix} 0.143 & -0.034 \\ -0.034 & 0.015 \end{bmatrix} \quad \lambda = \begin{bmatrix} \lambda_1 = 5.61 \\ \lambda_2 = 9.55 \end{bmatrix} \quad (37)$$

corresponding to the following damping ratio and natural frequency:

$$\zeta = 0.91 \quad \omega_n = 3.09 \text{ rad/h} \quad (38)$$

286 satisfying the performance constraints regarding the settling time  $T_s = \frac{4}{\zeta\omega_n} = 1.43$  h.

287 Figures 8 and 9 show the closed-loop response of biomass, substrate, acetate concentrations, and the corresponding feed flow-rate in 50 different runs, with  $A_{ref} = 0.5$  g/L  
 288 and kinetic parameter deviations. A white noise is added to the acetate concentration  
 289 measurement with zero mean and a standard deviation of 0.1 g/L. In all the runs, biomass  
 290 follows a similar exponential growth in the first hours, while the model errors show their  
 291 effect in the final hours. Nevertheless, the model uncertainties have a minor influence on  
 292 the controller performance as can be observed in the acetate evolution, where the set-  
 293 point is regulated and robust convergence is achieved by the controller. The noisy acetate  
 294

295 signal has a mean value of 0.49 g/L and the tracking error ( $A_{ref} - A$ ) has a RMSE of  
 296 0.0314 g/L which is lower than the measurement noise amplitude (0.1 g/L). Note that the  
 297 maximal 2h settling time condition is also satisfied. The biomass productivity remains  
 298 higher than 90 % of the nominal value in 90% of the runs, which is satisfactory from an  
 299 operational point of view.

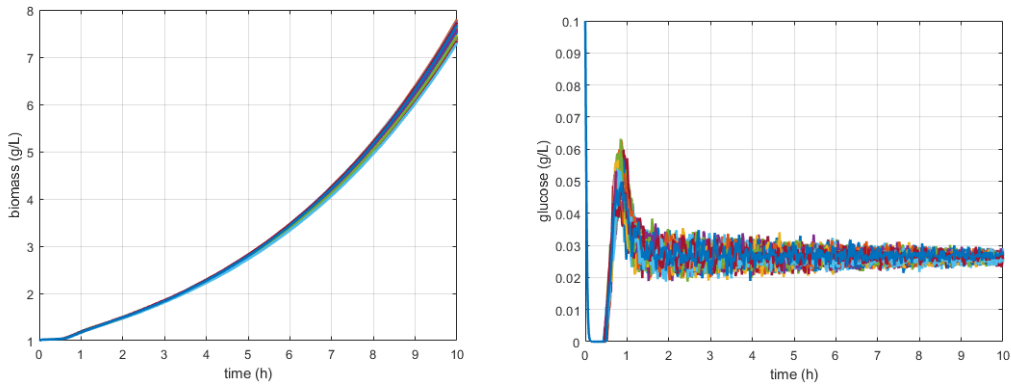


Figure 8: Biomass and substrate concentrations in 50 runs with kinetic parameter deviations (up to 15%) and a measurement noise standard deviation of 0.1 g/L using the robust GMC control strategy.

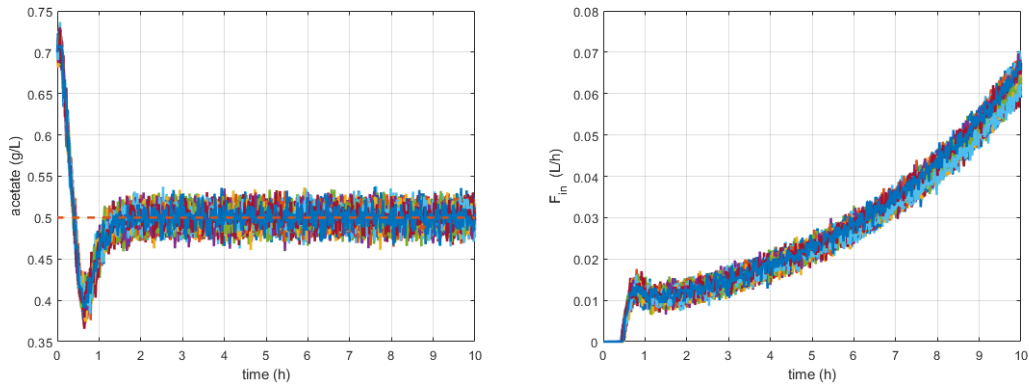


Figure 9: Acetate concentration and feed flow-rate in 50 runs with kinetic parameter deviations (up to 15%) and a measurement noise standard deviation of 0.1 g/L using the robust GMC control strategy.

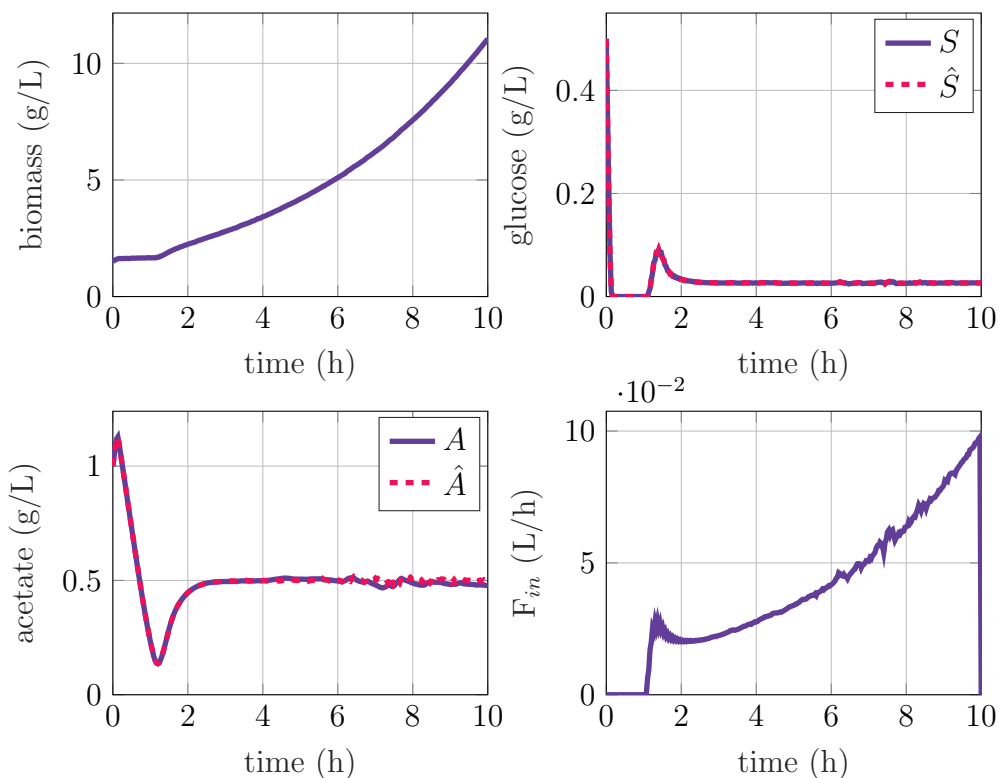


Figure 10: Coupled UKF-GMC with random parameter values ( $\pm 15\%$  variation) and a white measurement noise (std = 0.1 g/L).

300 Finally, the UKF and robust GMC are coupled, and their overall performance assessed  
 301 in a new set of numerical simulations. The UKF initial conditions are selected randomly  
 302 with a maximum deviation of 20% from the real values. Kinetic parameter variations of  
 303  $\pm 15\%$  of the nominal values and a white measurement noise with a standard deviation of  
 304 0.1 g/L are considered. As shown in Figure 10, the UKF behaves very well and converges  
 305 in the first hours to the real state trajectories. We can observe small estimation errors  
 306 with peaks and troughs around the real substrate value when the substrate (glucose)  
 307 concentration reaches a critical level of  $S_{crit}$ . Fortunately, this is not too detrimental for  
 308 the controller which is still able to track the acetate concentration reference set-point.

In order to test further the performance and robustness of the control approach, a comparison is achieved with the classical GMC algorithm presented in [22]. The parameter tuning is performed by selecting a desired rise time. In the presented simulations the following parametrization is chosen:

$$\xi = 1, \quad t_r = 2 \text{ h}, \quad \lambda_1 = 3, \quad \lambda_2 = 2.25$$

309 The classical and the robust controllers are tested in the ideal model case (no param-  
 310 eter variation), and in the case of a random variation in all model parameters up to 30%

311 of their nominal value. A series of 100 Monte Carlo (MC) simulations is performed and  
 312 the results are summarized in Table 2.

313 The results of one simulation are shown in Figure 11, where both approaches perform  
 314 similarly in the ideal model case. However, we can see that with increasing levels of  
 315 parameter variation, the robust GMC performs better in terms of reference tracking.  
 316 The mean square errors ( $\overline{e_A}$ ) and the mean acetate concentration ( $\overline{A}$ ) show that the  
 317 robust tuning of the parameters allows the controller to achieve the control objective  
 318 accurately. We can also observe that a 30% variation is the breakpoint of both methods,  
 319 with a slight advantage to the robust GMC design.

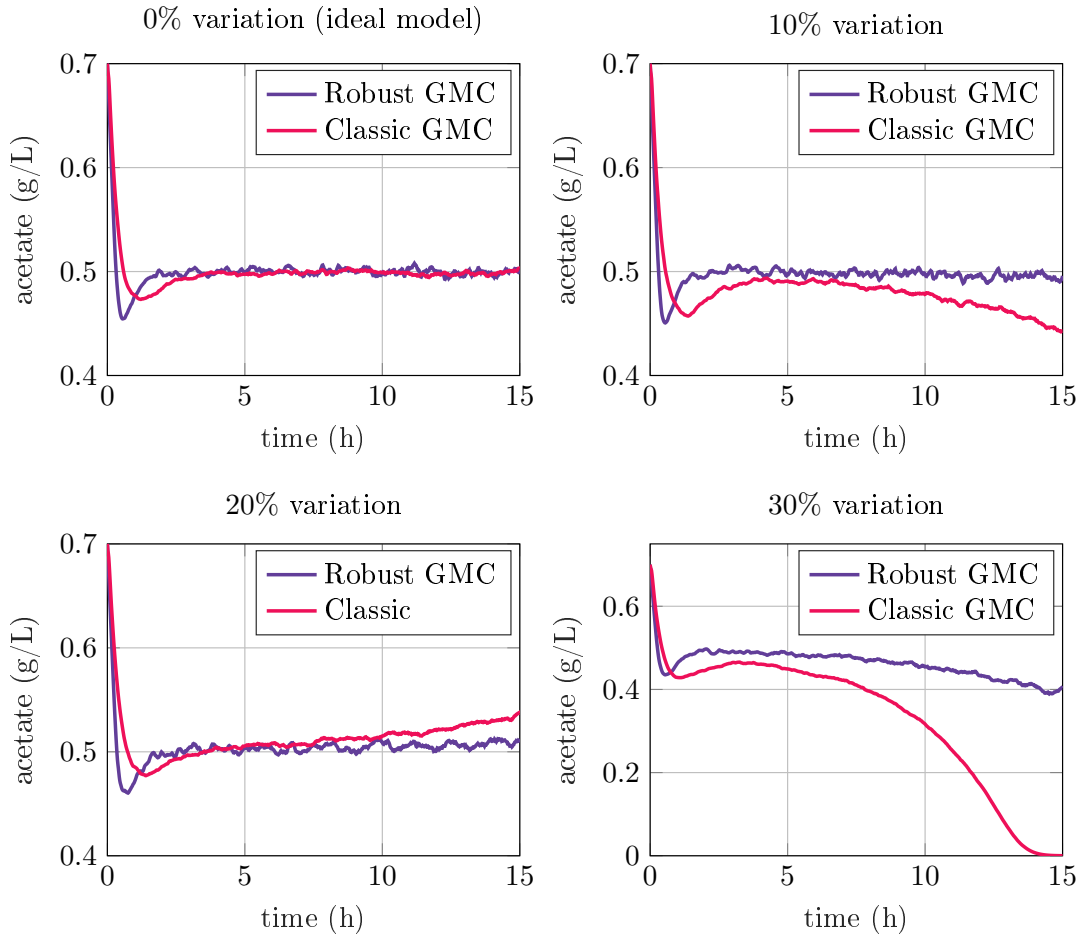


Figure 11: Comparison between the classical and robust tuning of the GMC strategy, with increasing levels of parameter variation.

Table 2: Results of 100 Monte Carlo simulations comparing the classical and robust GMC strategies

	$\bar{A}$ (Classic)	$\bar{A}$ (Robust)	$\bar{e}_A$ (Classic)	$\bar{e}_A$ (Robust)
0%	0.4996	0.4996	0.0226	0.0192
10%	0.4968	0.4989	0.02	0.0293
15%	0.5009	0.5001	0.0343	0.0207
20%	0.4992	0.4998	0.0456	0.0224
25%	0.4885	0.4974	0.0643	0.0261
30%	0.4744	0.4944	0.0843	0.0294
35%	0.4741	0.4913	0.0889	0.0418

## 320 7. Materials and methods

321 This section describes the strain, materials, growth media, and preparation methods  
 322 utilized in the experiments.

### 323 7.1. Microbial strain

324 The *E. coli* BL21(DE3) strain was used for all fermentations. BL21 is known to lead  
 325 to a low acetate formation compared to *E. coli* K12, which is suitable for high cell density  
 326 cultivations, as well as lower sensitivity to varying growth conditions [41].

### 327 7.2. Growth media and culture conditions

328 The media were prepared according to the protocol cited in [10, 23]. The media used  
 329 during the different stages of the cultures are the Lysogeny broth medium (LB) and a  
 330 defined high-density fermentation medium (HDF) [23]. Their respective compositions  
 331 for the batch (precultures & bioreactor) and fed-batch cultures are given in Tables 3  
 332 and 4. During the preparation, solutions were filtered and sterilized in autoclave to avoid  
 333 contamination.

Table 3: Composition of the LB media used during preparations

<b>Component</b>	<b>Concentration</b> (g/L)
Peptone	10
Yeast extract	5
NaCl	6

Table 4: Composition of the defined HDF media

<b>Components</b>	<b>Batch medium (./L)</b>	<b>Feeding solution (./L)</b>
Glucose	5 g	500.0 g
KH <sub>2</sub> PO <sub>4</sub>	13.3 g	–
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	4.0 g	–
MgSO <sub>4</sub> · 7H <sub>2</sub> O	1.2 g	20.0 g
Citric acid	1.7 g	–
EDTA	8.4 mg	13.0 mg
CoCl <sub>2</sub> · 6H <sub>2</sub> O	2.5 mg	4.0 mg
MnCl <sub>2</sub> · 4H <sub>2</sub> O	15.0 mg	23.5 mg
CuCl <sub>2</sub> · 4H <sub>2</sub> O	1.5 mg	2.5 mg
H <sub>3</sub> BO <sub>3</sub>	3.0 mg	5.0 mg
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	2.5 mg	4.0 mg
Zn (CH <sub>3</sub> COO) <sub>2</sub> · 2H <sub>2</sub> O	13.0 mg	16.0 mg
Fe <sup>III</sup> Citrate	100.0 mg	40.0 mg
Thiamine ·HCl	4.5 mg	–

### 334 7.3. Reactor setup

335 The cultivations were performed in a bioreactor consisting of a 5L jacketed glass  
336 vessel and a digital control unit or *DCU* (BIOSTAT B plus, Sartorius Stedim Biotech,  
337 Germany). The reactor is equipped with a water jacket and an agitation motor.

338 The monitoring of the cultures is possible thanks to a potentiometric pH sensor  
339 (Hamilton, Switzerland), optical dissolved oxygen (DO) probe (Hamilton, Switzerland),  
340 and a temperature sensor (Sartorius, Germany). Also, biomass concentration is available  
341 on-line via an absorption-based photometric turbidity probe (Fundalux II, Sartorius, Ger-  
342 many).

### 343 7.4. Analytical Methods

344 During the fermentation, samples were taken every hour. The optical density (OD) of  
345 the samples was measured at 600 nm in a UV spectrophotometer (Shimadzu, Pharmacia  
346 Biotech, USA). Samples were diluted with deionized water to obtain OD in the linear  
347 range (0-0.3 OD), and then correlated with dry cell weight (DCW) using a calibration  
348 curve to obtain the off-line biomass concentration. To determine dry cell weights (DCW),  
349 10 mL aliquots of culture medium were filtered and placed in pre-weighed polystyrene  
350 micro weighing dishes, dried at 65°C for 24 h until constant weight, and weighed (1 OD  
351 = 0.39 g/L).

352 Samples were centrifuged and the supernatant was decanted and then stored at -8  
353 °C. Glucose concentration was measured using the Dinitro Salicylic Acid (DNS) Method.

354 Acetate determination was performed using an enzymatic acetic acid test kit (Megazyme,  
355 Ireland). The sensitivity level of analytical methods is estimated around 0.1 g/L [30].

### 356 7.5. Operating conditions

357 The batch and fed-batch fermentations were conducted under controlled conditions.  
358 The pH is regulated at 7 by adding solutions of 12.5% ammonium hydroxide (base)  
359 or phosphoric acid 0.5 M (acid). Dissolved oxygen ( $pO_2$ ) was maintained above 30%  
360 air saturation by a two-level controller, increasing the agitation rate when the oxygen  
361 demand of the cells increases. When the maximal agitation rate is reached, the airflow  
362 is increased. Minimum values for airflow and agitation were imposed (1 L/min and 200  
363 rpm, respectively).

364 The temperature was controlled by the DCU at 37 °C using a heating water jacket.

### 365 7.6. Pre-cultures

366 The cryogenic culture was incubated for 24h on LB-agar Petri dishes at 37°C. Primary  
367 inocula consisting of Lysogeny broth (LB) media (Table 3), and one colony of *E. coli* were  
368 prepared in 150-mL Erlenmeyer shake flasks (50 mL working volume) and grown 8h at  
369 37°C in an air shaker at 250 rpm.

370 To adapt cell populations to fermentor conditions, shake flasks containing the HDF  
371 media (Table 4) (250 mL) were inoculated from the LB media cultures (5% v/v) and  
372 incubated overnight (14-16h) at 37°C in the air shaker at 200 rpm.

### 373 7.7. Batch and fed-Batch cultures

374 All fermentations were carried in batch, followed by a fed-batch phase. Initial batch  
375 cultures of  $V = 3.5$  L were pre-equilibrated to the appropriate operating conditions (pH,  
376  $pO_2$  temperature) before inoculation with 5% v/v seed culture, where the initial  $OD_{600}$   
377 in the fermentor reaches 0.3-0.6. It is noteworthy that a lower initial volume was not  
378 possible, since the biomass probe would not be completely immersed. Sterile filtered  
379 anti-foam was added via a peristaltic pump when necessary throughout the cultivations.

380 The batch phase was monitored during the day. Once the glucose was nearly depleted,  
381 the fed-batch phase started, and the feeding solution was added with a rate determined  
382 by the controller, and applied by a Reglo-digital peristaltic pump (Ismatec, Germany).

### 383 7.8. Culture monitoring and control

384 The measurements provided by the DCU ( $pH$ ,  $pO_2$ , Temperature ...) were monitored  
385 in real-time by the MFCS software (Sartorius, Germany). The control and estimation  
386 algorithms were coded in Matlab for simulations and implemented on-line in LabView  
387 using shared-library and MathScript nodes. Measurements provided by MFCS were im-  
388 ported to LabView by shared libraries. The biomass signal was measured separately by  
389 a data acquisition device from national instruments (NI USB-6000USB Multifunction

390 DAQ Device, National Instruments, USA) and squired in LabView using DAQMax li-  
 391 brary. Figure 12 shows a diagram of the different devices used for online implementation.

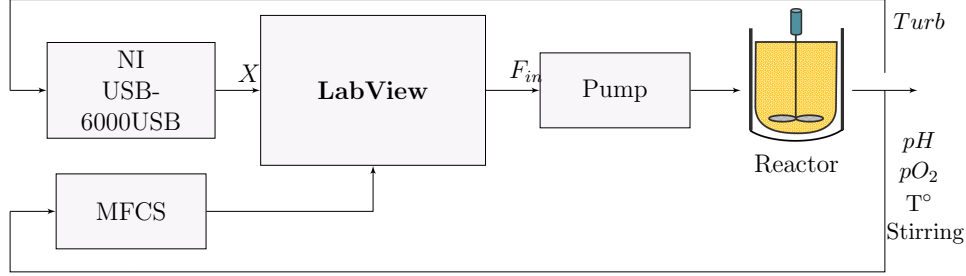


Figure 12: Real-time implementation diagram

## 392 8. Experimental results and discussion

393 Two control experiments were performed to test the tracking performance and robust-  
 394 ness of the developed UKF-GMC strategy in a real-time environment. Each experiment  
 395 consisted of a batch phase followed by a fed-batch phase (control phase). The evolution of  
 396 the measured biomass (on-line & off-line), glucose, acetate concentrations (off-line), and  
 397 their estimates, as well as the feed flow-rate (controller output), are shown in Figures 13  
 398 and 15. The operating conditions are also illustrated in Figures 14 and 16.

### 399 8.1. Culture evolution

400 After reaching the desired operating conditions, the reactor is inoculated with the seed  
 401 culture, and the batch phase begins. As shown in Figures 13 and 15, the initial biomass  
 402 concentration in the reactor ranges from 0.1-0.2 g/L. During this phase, the biomass  
 403 follows an exponential growth and reaches up to 2 g/L. Since glucose consumption leads  
 404 to acetate production, the culture is in respiro-fermentative mode.

405 The batch phase ends after 4-5 h, after the almost complete consumption of the glucose  
 406 in the medium. On-line indicators of the glucose depletion are the sudden decrease of the  
 407 stirring speed due to the decrease of cell demand for oxygen, the sudden increase of the  
 408 pH combined with the decrease of injected base volume (see Figures 14 and 16). Note  
 409 that the estimation algorithm is launched during the batch phase. The initial conditions,  
 410 control parameters, and acetate references for each experiment, as well as the values for  
 411 the measurement and process noise covariance matrices are given in Table 5,.

412 The fed-batch phase starts right after the first on-line flags and preferably before  
 413 complete depletion of glucose, to avoid the switch to the respirative mode. The GMC  
 414 controller is launched after setting up the acetate reference and the control parameters.  
 415 The feed solution is injected and the cells resume their growth, resulting in an increase  
 416 of the stirring speed due to the glucose oxidation, and the decrease of pH due to CO<sub>2</sub>  
 417 emission which requires base addition to maintain the pH around its set-point. The



418 fed-batch phase continues until reaching the saturation limit of the turbidimetric probe  
 419 (around 7-8 g/L). The maximum attainable cell density depends on the oxygenation  
 420 limitation related to the bioreactor scale as can be observed in several studies [10, 28,  
 421 42]. Therefore the end of the fed-batch phase is forced by either an exhausted feed  
 422 medium, or the limiting oxygenation conditions.

Table 5: Control & estimation parameters and initial conditions used in the experiments

	<b>Experiment 1</b>	<b>Experiment 2</b>
Sampling time	$t_e = 0.05 h$	$t_e = 0.05 h$
Acetate reference	$A_{ref} = 0.5 \text{ g/L}$	$A_{ref} = 0.7 \text{ g/L}$
$Q$	$diag[10^{-4}, 10^{-2}, 10^{-2}, 10^{-8}] \text{ g/L}$	$diag[10^{-4}, 10^{-2}, 10^{-2}, 10^{-8}] \text{ g/L}$
$R$	$10^{-4} \text{ g/L}$	$10^{-4} \text{ g/L}$

### 423 8.2. Acetate and glucose estimation

424 As presented in the simulation section, the on-line biomass concentration measurement  
 425 provided by the turbidimetric probe, and the kinetic model with identified parameter  
 426 values from [28] are used to estimate the acetate and glucose concentrations using the  
 427 UKF. The estimation is launched during the batch phase (around 4h).

428 The measurement noise affecting the biomass concentration signal is considered as a  
 429 centered white noise with a standard deviation of 0.1 g/L. On the other hand, the degree  
 430 of confidence in the model regarding the substrate and acetate concentration signals is  
 431 lower compared to the biomass concentration.

432 In both experiments, the UKF performance in the fed-batch phase is satisfactory,  
 433 despite the initialization errors and the model uncertainties. The glucose and acetate  
 434 estimations fit very well with the off-line measurements, and the convergence is achieved  
 435 in less than 1 h. Table 6 shows the estimation mean square error values for each estimated  
 436 state (i.e., substrate and acetate ) during the fed-batch phase of both experiments, which  
 437 are lower than the measurement sensitivity (0.1 g/L).

Table 6: Experimental study - UKF estimation mean square errors (in g/L)

	$\overline{e_S}(\text{g/L})$	$\overline{e_A}(\text{g/L})$
<b>Experiment 1</b>	0.0885	0.0679
<b>Experiment 2</b>	0.0381	0.1132

### 438 8.3. GMC control performance

439 The control objective, as explained in previous sections, is to regulate the acetate con-  
 440 centration to a predefined set-point, and maintain the culture in the respiro-fermentative

441 mode close to the optimal limit. As can be seen in Figures 13 and 15, acetate accumu-  
 442 lation is avoided in both cultures, and the concentration is limited to less than (1 g/L)  
 443 during the fed-batch phase.

444 In the first experiment (Figure 13), the estimated acetate concentration is regulated  
 445 and converges to the desired reference, respecting the chosen settling time. The second  
 446 experiment (Figure 15) presents the same performance regarding the GMC algorithm  
 447 convergence, with a different set-point and a longer control time.

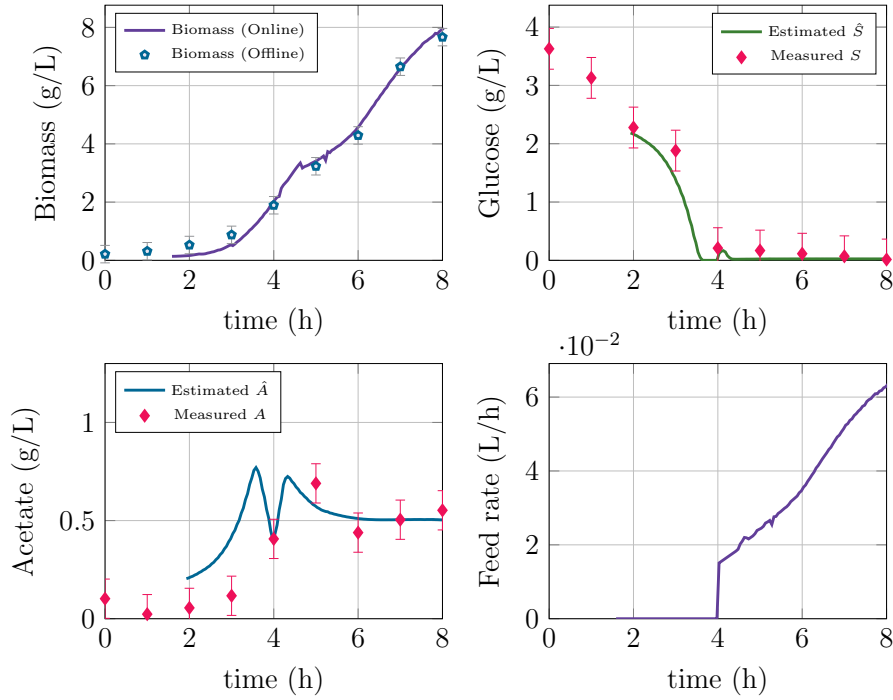


Figure 13: Experiment 1: Time evolution of the measured biomass, glucose, acetate concentrations estimates, and feed-rate

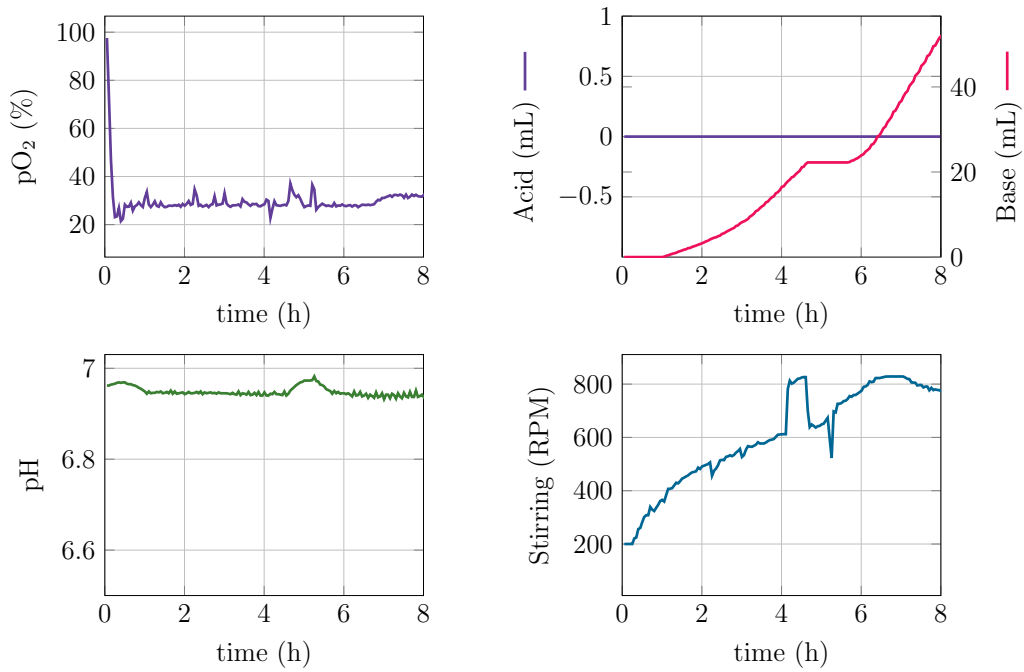


Figure 14: Experiment 1: Time evolution of the  $pO_2$ , acid and base concentrations, pH and stirring

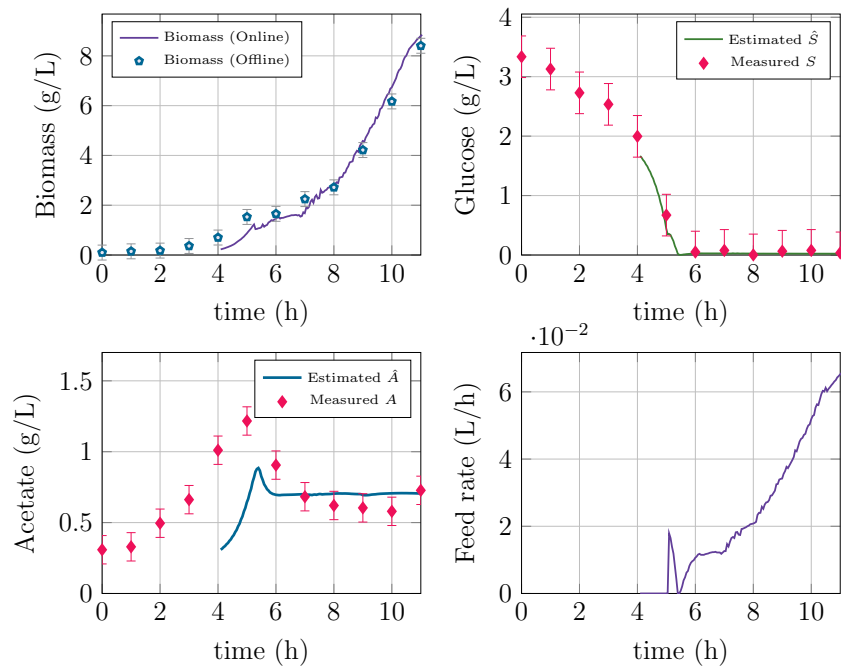


Figure 15: Experiment 2: Time evolution of the measured biomass, glucose, acetate concentrations, and feed-rate

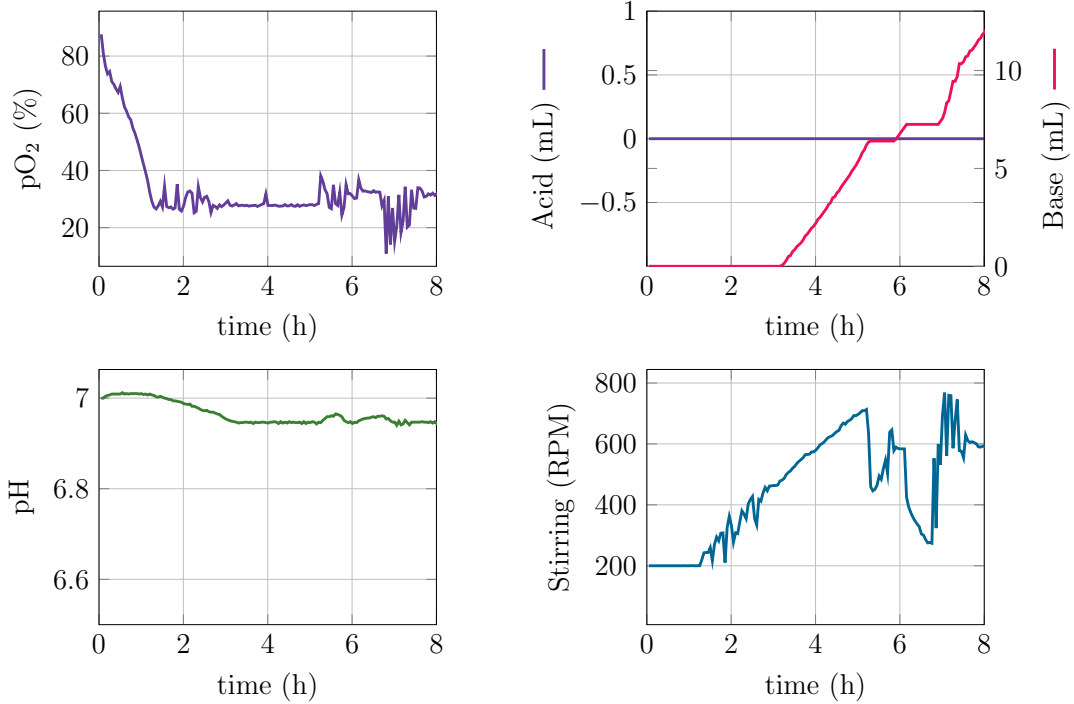


Figure 16: Experiment 2: Time evolution of the  $pO_2$ , acid and base concentrations, pH and stirring

#### 448 8.4. Discussion

449 The presented control method provides a practical approach to avoid overflow metabolism  
 450 in *E. coli* fed-batch cultures. However, it offers a suboptimal solution, since accurate on-  
 451 line measurements of the substrate concentration at the critical level are impractical.

452 In order to evaluate the efficiency of the proposed approach, a comparison is performed  
 453 with another suboptimal regulation strategy. In this approach, the growth rate is regu-  
 454 lated to a set reference value  $\mu_{set}$ , usually chosen slightly below the maximal growth rate  
 455 in order to avoid acetate accumulation while maximizing the biomass productivity. This  
 456 control objective is attained by tracking a predefined biomass trajectory corresponding  
 457 to the chosen reference growth rate [43, 44].

458 In this study, we compare the robust acetate regulation to an adaptive GMC strategy  
 459 tracking a defined growth rate presented in [19]. For this purpose, we set the biomass  
 460 regulation to track a defined growth rate  $\mu_{set}$  chosen at 90% of the theoretical maximal  
 461 value ( $\mu_{max} = 0.26$  L/h), corresponding to the critical substrate concentration and the  
 462 maximal oxidative capacity. On the other hand, we set the acetate regulation to track a  
 463 reference of 0.5 g/L.

464 First, we assume that the model parameters and maximal growth rate  $\mu_{X_{max}}$  are  
 465 perfectly known. Then, we introduce a fixed variation in the maximal oxidative capacity  
 466  $q_{O_{max}}$ , leading to a variation in the maximal growth rate.

467 Simulation results are shown in Figures 17 and 18. In the ideal model case (no pa-  
 468 rameter variation), the biomass growth regulation (GMC-*X*) has a slightly better overall  
 469 performance. The reference growth rate is tracked accurately at  $0.23 \text{ h}^{-1}$  corresponding  
 470 to 89% of its maximal value. On the other hand, regulating the acetate concentration  
 471 (GMC-*A*) at  $0.5 \text{ L/h}$  leads to a biomass growth rate of  $0.21 \text{ h}^{-1}$  corresponding to 81%  
 472 of the maximal value as can be seen in Table 7. This result shows that the presence  
 473 of acetate in the medium reduces the biomass growth rate, due to lower substrate con-  
 474 sumption rate caused by the activation of the acetate consumption pathways according  
 475 to the bottleneck theory. However, keeping the acetate at a low concentration reduces  
 476 its inhibitory effect, and keeps the culture close to the optimal conditions.

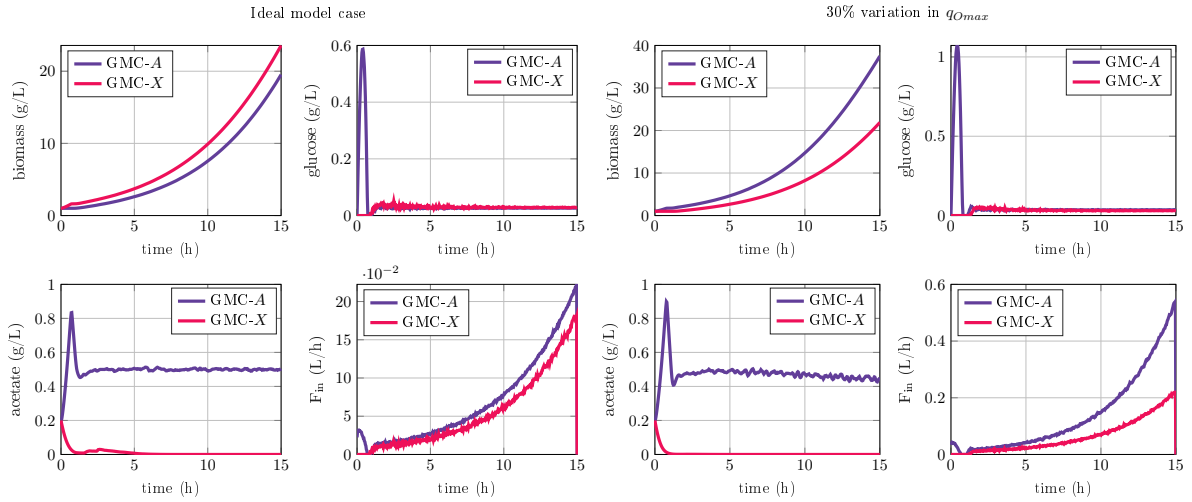


Figure 17: Comparison between the control approaches in the ideal model case, and in the presence of parametric variation. Plot of the state variables and the feed-rate.

Table 7: The effect of parameter variation on the control performance

Variation in $q_{Omax}$	$\frac{\mu_X}{\mu_{max}}$ % (GMC- <i>X</i> )	$\frac{\mu_X}{\mu_{max}}$ % (GMC- <i>A</i> )	$S_{crit}$
0%	89%	81%	0.0375
10%	81%	85%	0.046
20%	75%	89%	0.0529
30%	70%	93%	0.0628

477 The introduction of a 20% variation in  $q_{Omax}$  leads to an increase of the critical  
 478 substrate concentration  $S_{crit}$  and consequently the maximal growth rate  $\mu_{max}$ .

479 Despite the model mismatch, the biomass growth rate regulation presents a good  
 480 performance in tracking the reference rate. However, it corresponds to only 75% of the

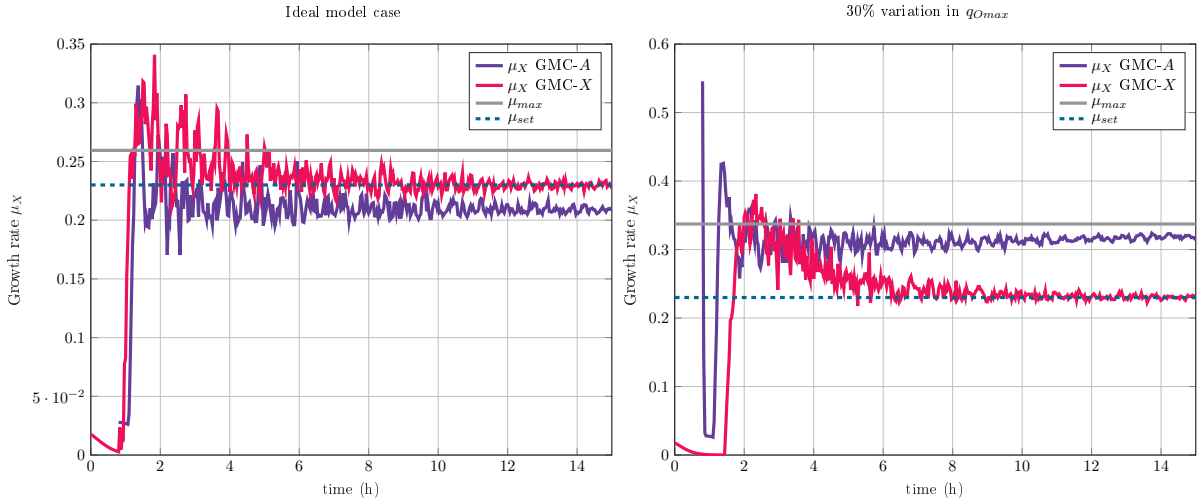


Figure 18: Comparison between the control approaches in the ideal model case, and in the presence of parametric variation. Plot of the specific biomass growth rates.

481 new maximal value, and therefore the biomass productivity is also lower than its optimal  
 482 value compared to the nominal case. The acetate regulation on the other hand offers a  
 483 more consistent performance, and gives a better growth rate ratio (89%). Furthermore,  
 484 the growth rate ratio is higher with increasing variation in the maximal oxidative capacity  
 485 as can be seen in Table 7. This result highlights a problem with targeting a specific  
 486 growth rate as a control objective, as it requires accurate determination of the maximal  
 487 value. This is a difficult task due to the uncertain nature of bioprocesses, as parameter  
 488 variation depends on several factors such as the variation in operating conditions between  
 489 batches. If the maximal growth rate is underestimated, the resulting suboptimal biomass  
 490 productivity is lower than the desired one. If the maximal growth rate is overestimated, a  
 491 regulation at 90% of this value could lead to acetate accumulation and metabolic switches,  
 492 and thereby a growth inhibition.

493 On the other hand, regulating the acetate concentration and maintaining it at a  
 494 low value offers a better practical trade-off, since the accumulation is avoided, and the  
 495 obtained growth rate is consistent in the case of model mismatch. This is an interesting  
 496 result since the acetate regulation approach is robust towards the change in operating  
 497 conditions, and is not specific to the bacterial strain. The strategy could be applied to a  
 498 different strain while ensuring the same level of performance without the need to estimate  
 499  $\mu_{max}$  accurately.

## 500 9. Conclusions

501 In this paper, a robust Generic Model Control scheme is presented and applied to  
 502 regulate the acetate concentration in *E. coli* BL21 (DE3) fed-batch cultures. The pro-

503 posed controller is based on feedback input-output linearization of the nonlinear model  
504 dynamics. A mechanistic model based on overflow metabolism is considered in the de-  
505 sign phase. Due to the uncertain nature of the model, a robust design procedure using  
506 the  $\mathcal{LMI}$  formalism is carried out, to compensate the model mismatch, disturbances,  
507 and measurement noise. Performance constraints are also formulated with  $\mathcal{LMI}$ s to en-  
508 sure desired properties of the closed-loop transient response. The controller performance  
509 and robustness are validated through a series of simulations. The controller manages to  
510 stabilize the uncertain system near the optimal operating conditions despite unmodeled  
511 dynamics and external disturbances. Since the controlled variable (acetate) is not avail-  
512 able for on-line measurement, a state estimation algorithm is required and an Unscented  
513 Kalman Filter (UKF) is implemented. The UKF is tuned based on experimental data,  
514 and validated both in simulation runs and in real-time experimental conditions. Finally,  
515 fed-batch experiments with a lab-scale reactor are performed in order to validate the  
516 efficiency of the coupled GMC-UKF strategy in driving the cultures near the optimal  
517 operating conditions.

518 Although the presented approach is suboptimal, it provides a practical solution to  
519 avoid overflow metabolism, since accurate measurements of the substrate concentration  
520 at the critical level are not possible. Furthermore, the strategy is not restricted to the  
521 studied strain since accurate determination of the maximal growth rate is not required.  
522 It is also adaptable to different control objectives such as substrate regulation at high  
523 concentrations in order to promote the product formation.

524 An improvement of the proposed control scheme is tracking a successively decreasing  
525 set-point calculated by numerical on-line optimization based on the estimation of the  
526 maximal growth rate. An experimental validation of this approach in future works could  
527 improve the process productivity since it provides a good trade-off between practicality  
528 and best achievable sub-optimality.

## 529 References

- 530 [1] S. Pontrelli, T.-Y. Chiu, E. I. Lan, F. Y.-H. Chen, P. Chang, and J. C. Liao, “Es-  
531 cherichia coli as a host for metabolic engineering,” *Metabolic Engineering*, vol. 50,  
532 pp. 16–46, 2018.
- 533 [2] S. Y. Lee, “High cell-density culture of escherichia coli,” *Trends in Biotechnology*,  
534 vol. 14, no. 3, pp. 98–105, 1996.
- 535 [3] G. W. Luli and W. R. Strohl, “Comparison of growth, acetate production, and  
536 acetate inhibition of Escherichia coli strains in batch and fed-batch fermentations,”  
537 *Applied and Environmental Microbiology*, vol. 56, no. 4, pp. 1004–1011, 1990, ISSN:  
538 00992240.

- 539 [4] K. Han, H. C. Lim, and J. Hong, "Acetic acid formation in escherichia coli fermenta-  
540 tion," *Biotechnology and Bioengineering*, vol. 39, no. 6, pp. 663–671, 1992, ISSN:  
541 10970290.
- 542 [5] M. Van De Walle and J. Shiloach, "Proposed mechanism of acetate accumula-  
543 tion in two recombinant Escherichia coli strains during high density fermentation,"  
544 *Biotechnology and Bioengineering*, 1998, ISSN: 00063592.
- 545 [6] H. Crabtree, "Observations on the carbohydrate metabolism of tumours," *Biochem*  
546 *J*, vol. 23, pp. 536–545, 1929.
- 547 [7] D. Riesenberger, V. Schulz, W. Knorre, H.-D. Pohl, D. Korz, E. Sanders, A. Roß,  
548 and W.-D. Deckwer, "High cell density cultivation of escherichia coli at controlled  
549 specific growth rate," *Journal of Biotechnology*, vol. 20, no. 1, pp. 17–27, 1991.
- 550 [8] S. A. Rothen, M. Sauer, B. Sonnleitner, and B. Witholt, "Growth characteristics  
551 of escherichia coli hb101[pgec47] on defined medium," *Biotechnology and Bioengi-  
552 neering*, vol. 58, no. 1, pp. 92–100, 1998.
- 553 [9] B. W. Srinivasan, B. Sedghi, and D. Bonvin, "Terminal-cost optimization of a class  
554 of hybrid systems," *European Control Conference (ECC)*, vol. 09, pp. 3167–3172,  
555 2001, ISSN: 00992240.
- 556 [10] I. Rocha, "Model-based strategies for computer-aided operation of a recombinant  
557 e. coli fermentation," Ph.D. dissertation, Universidade do Minho, Braga, 2003.
- 558 [11] S. Valentinotti, B. Srinivasan, U. Holmberg, D. Bonvin, C. Cannizzaro, M. Rhiel,  
559 and U. von Stockar, "Optimal operation of fed-batch fermentations via adaptive  
560 control of overflow metabolite," *Control Engineering Practice*, 2003, ISSN: 09670661.
- 561 [12] S. Jana and J. K. Deb, "Strategies for efficient production of heterologous proteins  
562 in Escherichia coli," in *Applied Microbiology and Biotechnology*, vol. 67, Springer  
563 Verlag, 2005, pp. 289–298.
- 564 [13] X. Hulhoven, F. Renard, S. Dessoy, P. Dehottay, P. Bogaerts, and A. Vande Wouwer,  
565 "Monitoring and control of a bioprocess for malaria vaccine production," *IFAC Pro-  
566 ceedings Volumes*, vol. 39, no. 9, pp. 143–148, 2006.
- 567 [14] L. Dewasme, D. Coutinho, and A. Vande Wouwer, "Adaptive and robust lineariz-  
568 ing control strategies for fed-batch cultures of microorganisms exhibiting overflow  
569 metabolism," in *Lecture Notes in Electrical Engineering*, vol. 89 LNEE, Springer,  
570 Berlin, Heidelberg, 2011, pp. 283–305, ISBN: 9783642195389.
- 571 [15] L. Dewasme, B. Srinivasan, M. Perrier, and A. Vande Wouwer, "Extremum-seeking  
572 algorithm design for fed-batch cultures of microorganisms with overflow metabolism,"  
573 *Journal of Process Control*, vol. 21, no. 7, pp. 1092–1104, 2011.



- 574 [16] L. Santos, L. Dewasme, D. Coutinho, and A. Vande Wouwer, “Nonlinear model pre-  
575 dictive control of fed-batch cultures of micro-organisms exhibiting overflow metabolism:  
576 Assessment and robustness,” *Computers & Chemical Engineering*, vol. 39, pp. 143–  
577 151, 2012, ISSN: 0098-1354.
- 578 [17] K. Gonzalez, S. Tebbani, F. Lopes, A. Thorigné, S. Givry, D. Dumur, and D.  
579 Pareau, “Regulation of lactic acid concentration in its bioproduction from wheat  
580 flour,” *Control Engineering Practice*, vol. 54, pp. 202–213, 2016.
- 581 [18] M. Jenzsch, R. Simutis, and A. Luebbert, “Generic model control of the specific  
582 growth rate in recombinant escherichia coli cultivations,” *Journal of Biotechnology*,  
583 vol. 122, no. 4, pp. 483–493, 2006.
- 584 [19] M. Abadli, L. Dewasme, S. Tebbani, D. Dumur, and A. Vande Wouwer, “Generic  
585 model control applied to E. coli BL21(DE3) Fed-batch cultures,” *Processes*, vol. 8,  
586 no. 7, p. 772, Jul. 2020, ISSN: 22279717.
- 587 [20] G. L. Kleman and W. R. Strohl, “Acetate metabolism by Escherichia coli in high-  
588 cell-density fermentation,” *Applied and Environmental Microbiology*, 1994, ISSN:  
589 00992240.
- 590 [21] S. Pinhal, D. Ropers, J. Geiselmann, and H. De Jong, “Acetate metabolism and  
591 the inhibition of bacterial growth by acetate,” *Journal of Bacteriology*, 2019, ISSN:  
592 10985530.
- 593 [22] P. Lee and G. Sullivan, “Generic model control (gmc),” *Computers & Chemical*  
594 *Engineering*, vol. 12, no. 6, pp. 573–580, 1988.
- 595 [23] M. DeLisa, H. Chae, W. Weigand, J. Valdes, G. Rao, and W. Bentley, “Generic  
596 model control of induced protein expression in high cell density cultivation of es-  
597 cherichia coli using on-line gfp-fusion monitoring,” *Bioprocess and Biosystems En-*  
598 *gineering*, vol. 24, no. 2, pp. 83–91, Sep. 2001.
- 599 [24] D. J. Costello, P. L. Lee, and P. F. Greenfield, “Control of anaerobic digesters using  
600 Generic Model Control,” *Bioprocess Engineering*, 1989, ISSN: 0178515X.
- 601 [25] P. L. Douglas, P. S. Fountain, G. R. Sullivan, and W. Zhou, “Model based control of  
602 a high purity distillation column,” *The Canadian Journal of Chemical Engineering*,  
603 1994, ISSN: 1939019X.
- 604 [26] I. Rocha and E. Ferreira, “Model-based adaptive control of acetate concentration  
605 during the production of recombinant proteins with e. coli,” *IFAC Proceedings Vol-*  
606 *umes*, vol. 35, no. 1, pp. 461–466, 2002, 15th IFAC World Congress.
- 607 [27] G. Bastin and D. Dochain, “On-line estimation and adaptive control of bioreactors,”  
608 *Analytica Chimica Acta*, 1991, ISSN: 00032670.

- 609 [28] C. Retamal, L. Dewasme, A. L. Hantson, and A. Vande Wouwer, “Parameter es-  
610 timation of a dynamic model of Escherichia coli fed-batch cultures,” *Biochemical*  
611 *Engineering Journal*, vol. 135, pp. 22–35, Jul. 2018, ISSN: 1873295X.
- 612 [29] B. Sonnleitner and O. Käppeli, “Growth of saccharomyces cerevisiae is controlled  
613 by its limited respiratory capacity: Formulation and verification of a hypothesis,”  
614 *Biotechnology and Bioengineering*, vol. 28, no. 6, pp. 927–937, 1986.
- 615 [30] L. Dewasme, G. Goffaux, A. L. Hantson, and A. Vande Wouwer, “Experimental  
616 validation of an Extended Kalman Filter estimating acetate concentration in E.  
617 coli cultures,” in *Journal of Process Control*, vol. 23, Feb. 2013, pp. 148–157.
- 618 [31] E. Peter and L. Lee, *Nonlinear Process Control: Applications of Generic Model*  
619 *Control*. London Ltd. UK: Springer-Verlag, 1993.
- 620 [32] A. Isidori, M. Thoma, E. D. Sontag, B. W. Dickinson, A. Fettweis, J. L. Massey,  
621 and J. W. Modestino, *Nonlinear Control Systems*, 3rd. Berlin, Heidelberg: Springer-  
622 Verlag, 1995, ISBN: 3540199160.
- 623 [33] W. Zhou, P. L. Lee, and G. R. Sullivan, “Robust stability analysis of generic model  
624 control,” *Chemical Engineering Communications*, vol. 117, no. 1, pp. 41–72, 1992.
- 625 [34] M. Chilali and P. Gahinet, “ $H_\infty$  design with pole placement constraints: An LMI  
626 approach,” *IEEE Transactions on Automatic Control*, 1996, ISSN: 00189286.
- 627 [35] R. Wood, “Automatic control systems,” *Water Research*, 1972, ISSN: 00431354.
- 628 [36] P. Bogaerts and A. Vande Wouwer, “Parameter identification for state estimation  
629 - Application to bioprocess software sensors,” *Chemical Engineering Science*, 2004,  
630 ISSN: 00092509.
- 631 [37] S. J. Julier and J. K. Uhlmann, “New extension of the Kalman filter to nonlinear  
632 systems,” in *Signal Processing, Sensor Fusion, and Target Recognition VI*, 1997,  
633 ISBN: 0819424838.
- 634 [38] —, “Unscented filtering and nonlinear estimation,” in *Proceedings of the IEEE*,  
635 2004.
- 636 [39] L. Dewasme, S. Fernandes, Z. Amriht, L. O. Santos, P. Bogaerts, and A. Vande  
637 Wouwer, “State estimation and predictive control of fed-batch cultures of hybridoma  
638 cells,” *Journal of Process Control*, vol. 30, pp. 50–57, 2015, ISSN: 09591524.
- 639 [40] J. F. Sturm, “Using sedumi 1.02, a matlab toolbox for optimization over symmetric  
640 cones,” *Optimization methods and software*, vol. 11, no. 1-4, pp. 625–653, 1999.
- 641 [41] M. Müller, W. Meusel, U. Husemann, G. Greller, and M. Kraume, “Application of  
642 heat compensation calorimetry to an E. coli fed-batch process,” *Journal of Biotech-*  
643 *nology*, vol. 266, pp. 133–143, Jan. 2018, ISSN: 18734863.

- 644 [42] L. Dewasme, A. Richelle, P. Dehottay, P. Georges, M. Remy, P. Bogaerts, and A.  
645 Vande Wouwer, “Linear robust control of *s. cerevisiae* fed-batch cultures at different  
646 scales,” *Biochemical Engineering Journal*, vol. 53, no. 1, pp. 26–37, 2010.
- 647 [43] I. Rocha, A. Veloso, S. Carneiro, E. Ferreira, and R. Costa, “Implementation of a  
648 specific rate controller in a fed-batch *E. coli* fermentation,” in *IFAC Proceedings*  
649 *Volumes (IFAC-PapersOnline)*, 2008, ISBN: 9783902661005.
- 650 [44] H. De Battista, J. Picó, and E. Picó-Marco, “Nonlinear PI control of fed-batch pro-  
651 cesses for growth rate regulation,” *Journal of Process Control*, 2012, ISSN: 09591524.