Control of Specific Growth Rate in Fed-Batch Bioprocesses: Novel Controller Design for Improved Noise Management

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Abstract: Accurate control of the specific growth rate (μ) of microorganisms is dependent on the ability to quantify the evolution of biomass reliably in real time. Biomass concentration can be monitored online using various tools and methods, but the obtained signal is often very noisy and unstable, leading to inaccuracies in the estimation of μ. Furthermore, controlling the growth rate is challenging as the process evolves nonlinearly and is subject to unpredictable disturbances originating from the culture's metabolism. In this work, a novel feedforward-feedback controller logic is presented to counter the problem of noise and oscillations in the control variable and to address the exponential growth dynamics more effectively. The controller was tested on fed-batch cultures of *Kluyveromyces marxianus*, during which μ was estimated in real time from online biomass concentration measurements obtained with dielectric spectroscopy. It is shown that the specific growth rate can be maintained at different setpoint values with an average root mean square control error of 23 ± 6%.

1. Introduction

The field of biotechnology is experiencing continued progress, owing to advances in online process monitoring and control. The application of various process analytical technologies enables researchers and end users to have insight into the process and to monitor its substrate uptake, biomass evolution, and cell metabolism. Traditionally, cultures were mostly performed in batch mode. One of the main drawbacks of this mode is that the growth dynamics cannot be controlled during the culture [1,2]. Significant improvement in this respect can be achieved by switching to the fed-batch mode and putting in place appropriate process supervision methodologies. In addition to greater control over the product quality, the cost efficiency of the process increases along with the enhancement of the culture yield [1,3,4].

The key variable that can be controlled in a fed-batch culture is the specific growth rate, μ. This variable has a direct effect upon the cell metabolism, and controlling it can be used to supress or to induce, depending on the need, the formation of secondary metabolites [3,5–8]. The challenge to control the specific growth rate comes from the fact that the determination of its instantaneous

value is dependent on the online estimation of the biomass concentration. Indeed, there does not yet exist a direct online measurement tool for cell concentration. All the currently used technologies offer an indirect estimation, where the measured signal is correlated to the biomass concentration through an external calibration [9]. Two different monitoring approaches can be identified. The first approach is noninvasive towards the process and typically involves the use of off-gas analysis combined with a metabolic model to estimate the biomass concentration and its evolution [10–12]. The second approach, invasive to the process, includes the application of an in-situ optical density probe [13–15], dielectric (capacitance) spectroscopy [16–18], or fluorescence spectroscopy [19,20] to monitor biomass. Other methods rely on a significant capital investment, including biocalorimetry [21]. The main issue with the in-situ monitoring technologies is their sensitivity to the process conditions. In particular, aeration and agitation interfere with the probe, and thus create noise in the signal [16,19]. Noise and instabilities in the biomass concentration signal are directly propagated to the estimation of μ, making control of the latter a challenging task. For these reasons, biomass measuring technologies require appropriate data treatment methods, ranging from basic signal smoothing and baseline adjustment to complex filtering algorithms such as Kalman filter [22]. Signal filtering always involves a tradeoff between reducing noise and minimizing measurement delay which are both undesirable in process control applications.

With the appropriate tools set in place to estimate the cell concentration online, several methodologies are available to control the specific growth rate. The easiest approach is the application of a predetermined exponential feed. Here, the initial conditions of the fed-batch phase are used to calculate the feed rate profile corresponding to the specific growth rate setpoint, and a feedforward controller is used to implement the profile [1]. However, no feedback action is available to intervene in case the process does not follow the desired growth rate. Feedback control typically involves the use of the following two classical types of controllers: proportional (P) and proportional-integral (PI) (and, more rarely, proportional integral derivative (PID)). They are considered to be simple gains controllers since they are not based on a model [6,23–25]. Dabros et al. [24] showed that the use of a mixed, feedforward-feedback controller reduced the mean control error by 32% as compared with applying a purely feedforward exponential feed profile. As mentioned above, the noise present in the biomass measurement and in the μ estimation, in particular, is a challenge when using feedback controllers to regulate the specific growth rate. Both the proportional and the integral parts of the controller are predisposed to amplify the noise and induce undesirable oscillations to the process. Therefore, the data must be treated and smoothed prior to use [26].

More advanced controllers are often model-based, and include the popular model predictive controller (MPC) and artificial neural networks [1,2]. These control approaches require extensive process knowledge in order to build and validate a data-driven model [1,27,28], which can be difficult to achieve as part of a new project or cell strain. Recently, successful implementation of growth rate controllers based on MPC, where noninvasive Process Analytical Technology (PAT) tools were applied towards microbial cultures, has been reported [29–32]. In addition, multisensor techniques have been applied by combining dielectric capacitance monitoring with biocalorimetry to reduce measurement noise [33]. However, these approaches add an extra layer of process complexity, resulting either from the extra modeling effort required or from the application of additional PAT tools. A critical advantage of dielectric capacitance monitoring is that it offers a direct and easily scalable method of biomass estimation [34,35] that can also be applied to single-use bioreactors [36,37]. Indeed, the signal-to-noise ratio often increases with scale, due to the reduced effect of bubbles on the probe, and therefore fast process development from bench scale to pilot scale [34]. Thus, control applications that require process scale-up would benefit from the reduced process complexity by applying dielectric capacitance as compared with utilizing multivariate approaches or biocalorimetry monitoring, where significant investment is required on scale-up [38]. Additionally, as no modeling is involved to implement direct dielectric monitoring based control, the systems developed should be easily transferable to other organisms, as previously demonstrated [24,39].

The technique used to control the specific growth rate depends on the ability to monitor the biomass and to manage the nonlinear growth dynamics of the process. A simple exponential feed cannot cope with unforeseen metabolic changes or process variability, while P or PI feedback controllers can have issues dealing with the exponential nature of cellular growth and with signal noise. Ensuring reliable biomass monitoring and a robust way of estimating μ is essential, as is the proper design of a controller capable of addressing the particularities of the process.

In this work, a revised control logic is presented to counter the above-mentioned issues. Biomass concentration measurements, provided by a dielectric spectroscopy probe, are smoothed with the Savitzky–Golay algorithm and used to estimate the current specific growth rate of the process. A modified feedforward-feedback controller is designed and optimized to maintain μ at the desired setpoint. The novel control design is adapted to the culture's exponential dynamics, resulting in improved noise management.

2. Materials and Methods

2.1. Cell Strain and Culture Conditions

In this study, the fed-batch cultures were performed with the wild type strain of *Kluyveromyces marxianus* DSMZ 5422. This strain was chosen mainly for its fast growth dynamics and easy cultivation conditions. A cell bank of 24 cryovials was prepared from four colonies of *K. marxianus* isolated on a fresh YPLA (yeast extract 10 g/L, peptone 20 g/L, lactose 40 g/L, and agar 20 g/L) petri dish. Each colony was inoculated in a sterile tube with 5 mL of YPL (yeast extract 10 g/L, peptone 20 g/L, and lactose 40 g/L), previously autoclaved at 121 °C for 15 min, and cultivated overnight at 30 °C and 150 rpm. The 5 mL of media were transferred to a 500 mL conical flask containing 100 mL of the previously described YPL medium and cultivated overnight at 30 °C and 150 rpm. Then, the cultures were transferred into four sterile tubes of 25 mL and centrifuged at 3500 rpm for 3 min. The biomass deposited in the four tubes was resuspended in a 5.5 mL solution of NaCl 0.9% and peptone 0.1%, and a 5.5 mL solution of glycerol 40%, reaching a total volume of 12 mL. Finally, sterile cryovials were filled with 2 mL each and stored in the freezer at −18 °C.

All the precultures were inoculated with a frozen vial retrieved from the cell bank. The cells were grown for 24 h at 30 °C and 120 rpm in a 1000 mL baffled conical flask containing 200 mL of YPL solution cited above. The preculture media were autoclaved for 10 min at 115 °C. After 24 h, the cells were isolated by centrifugation at 3500 rpm for 3 min and resuspended in a 4.5 mL solution of NaCl 0.9% and peptone 0.1%, then transferred to a 10 mL sterile syringe for inoculation.

The cultures were performed at 30 °C in a 3.6 L bench scale bioreactor (KLF, BioEngineering AG, Wald, Switzerland), with a working volume of 2.5 L. The agitation rate was set to 1000 rpm and the air flow rate to 250 NL/h, corresponding to 2 vvm, to maintain the dissolved oxygen (DO) levels above 20% throughout the experiments. In addition, to accommodate the strains particularly high respiration demands, a sintered sparger was used to enhance oxygen transfer and obtain a $k_L a$ value of 0.06 s^{-1}. The bioreactor was equipped with a double 6-blade Rushton type agitator, a PT-100 temperature probe, pH and DO probes (Mettler-Toledo, Columbus, OH, USA), inlet gas flow controller, exhaust gas port, acid and base ports, a feed port, an antifoam port, and a sampling port, as illustrated in Figure 1. The exhaust gas passed through a condenser cooled to 4 °C to minimize liquid loss. Temperature, pH, and pO$_2$ were controlled using the software provided by BioEngineering. Medium pH was controlled at 5 using solutions of NaOH 3 M and HCl 1 M. A solution of 10% antifoam in water was used to prevent foam formation.
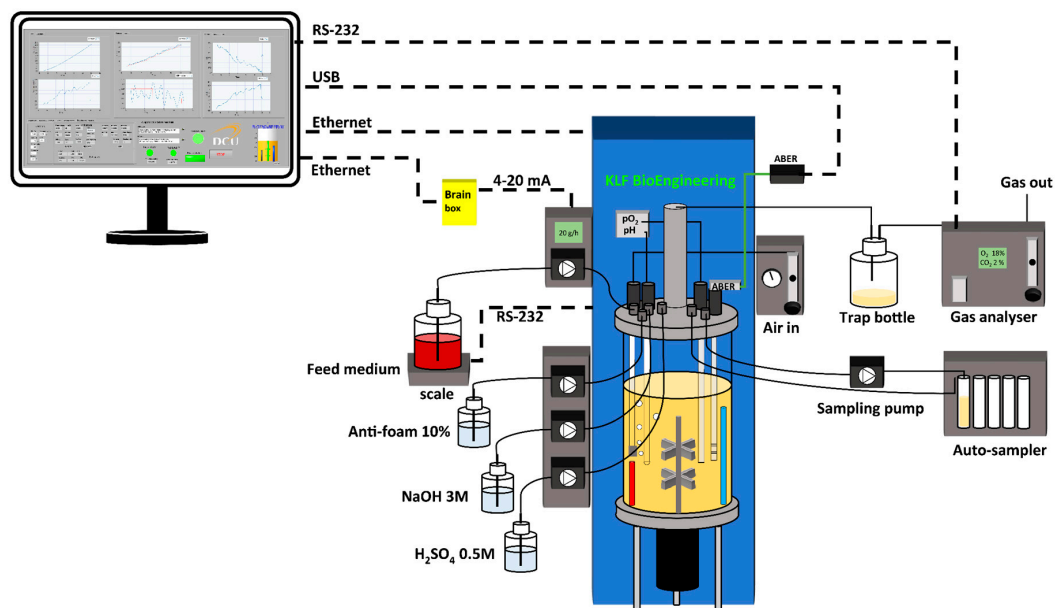
Figure 1. Fed-batch bioreactor setup.

Biomass concentration was monitored with a dielectric spectroscopy probe (ABER Instruments Ltd., Aberystwyth, UK). The probe was used in dual-frequency mode at 580 and 15,650 Hz with the polarization correction BM220 PolC tuned on and the filter value set at 140 to decrease the impact of aeration on the reading. Using the dual-frequency mode is more reliable because it minimizes the influence of baseline shifts and medium conductivity changes on the dielectric signal. Feeding was performed with a Peripex W2 pump (BioEngineering), which was controlled analogically between 4 and 20 mA by the current loop box ED-550 (Brainboxes, Liverpool, UK). Oxygen and CO_2 concentration levels in the exhaust gas were analyzed using a Tandem Pro gas analyzer (Magellan BioTech, Borehamwood, UK).

The cells were first grown in batch mode until depletion of the carbon source (which was detected by monitoring the dissolved oxygen and off-gas analysis signals), and then in fed-batch mode. Table 1 shows the semi-defined batch and feed media composition. Yeast extract was used as an iron and vitamin source [40–42], whereas peptone was used as an additional source of vitamins and protein, replacing a trace elements solution typically used [16,24,43].

Table 1. Composition of the batch and feed media.

Component	Batch Medium g/L	Feed Medium g/L
Lactose	5	300
$(NH_4)_2SO_4$	5	50
KH_2PO_4	3	25
$MgSO_4 \cdot 7H_2O$	0.5	3
Peptone	5	25
Yeast extract	5	25
Antifoam	2	3

2.2. Signal Filtering and Smoothing

A data acquisition program was developed using LabVIEW 18 (National Instruments, Austin, TX, USA) to handle all data communications between the KLF bioreactor and process analyzers (dielectric probe and off-gas analyzer). The program also treated the dielectric signal, reducing measured noise online. Basic moving average and the Savitzky–Golay smoothing algorithms were implemented and evaluated, with the latter showing better performance in terms of striking a compromise between

minimizing signal delay and maximizing the accuracy of the filtered signal. A window of 121 data points and a first-order polynomial fit was used within the Savitzky–Golay algorithm to smooth the dielectric signal ahead of the specific growth rate estimation.

2.3. Dielectric Spectroscopy Adaptive Calibration

The technology of dielectric spectroscopy, when used in dual-frequency mode, measures the drop in the medium permittivity between the two electrical field frequencies. This drop is assumed to be linearly proportional to the concentration of live cells; however, the correlation is different for each cell strain and culture conditions [44]. The magnetic field used by the sensor is sensitive to the average bubble size, medium conductivity and, to some extent, fluid viscosity. In order to maintain the sensor's response accuracy to these changing parameters, the probe was recalibrated for each culture using the cell concentration data acquired at the end of batch phase. The adapted calibration model was included within the LabVIEW on-line monitoring program prior to fed-batch phase, accounting for changes in medium conductivity and fluid viscosity, and applied for the fed-batch segment of the experiment.

For the purpose of reference, cell concentration was measured off-line by dry cell weight, at intervals of 60 min during the batch phase and 3 h during the fed-batch phases. Samples of 5 mL of broth were filtered through a prescaled membrane filter. Then, the filter was dried until constant weight, the yeast mass was calculated and converted to grams per litre.

2.4. Specific Growth Rate Estimation

The filtered biomass signal was used to calculate the estimated specific growth rate. As cell growth is an exponential process, the linearization involves the use of a logarithm within the derivative. The theoretical way of calculating μ is given in Equation (1), where C_x is the current biomass concentration and V the medium volume.

$$\mu(t) = \frac{d\ln(C_X V)}{dt} \tag{1}$$

Specific growth rate values obtained with Equation (1) are, however, very sensitive to any residual noise in the biomass measurement. In order to minimize this effect, a Δt window of 15 min was used in this work to calculate μ, as shown in Equation (2). This window size was the result of a compromise between reducing noise and minimizing signal delay.

$$\mu_{est}(t) = \frac{\ln\left(\frac{C_{X,t} V_t}{C_{X,t-15min} V_{t-15min}}\right)}{\Delta t} \tag{2}$$

Throughout the batch phase, the volume of the medium remained constant; however, during the fed-batch phase, the culture volume increased as a function of the feed flow rate. The instantaneous value of the volume was updated by monitoring the mass of medium fed into the reactor and taking into account media density. Equation (3) shows the calculation used to correct the medium volume, V_t, throughout the culture. Here V_{ini} is the initial volume of the batch medium, m_{fed} is the mass of the feed solution fed into the vessel, as recorded by the scale, and ρ_{feed} is the feed density.

$$V_t = V_{ini} + \frac{m_{fed}}{\rho_{feed}} \tag{3}$$

2.5. Controller Design

The classic method of regulating the specific growth rate is open-loop (feed-forward) control; this technology implies implementing a predefined, exponential feed rate of medium to the culture. However, using this approach does not allow reacting to any potential process disturbances or deviations from the setpoint. In order to enhance the open-loop action, a feedback logic can be

implemented [5,6,8]. Dabros et al. [24] proposed a new type of PI controller where the controller gains were included within the exponential term of the control equation. The advantage with this control logic is that it is well adapted to the exponential dynamics of the culture, making it more effective and robust over the course of the fed-batch culture.

In this work, we demonstrate the use of a similar feedforward-feedback controller, as the one proposed in [24], but with a slight modification. The proposed control logic alteration allows for greater noise management and makes the controller more robust, particularly for long-duration fed-batch cultures.

The feed-forward part of the controller action, for a given specific growth rate setpoint, μ_{sp}, is given by the following expression:

$$F_{FF}(t) = F_0 \exp\left(\mu_{sp}t\right) \tag{4}$$

where F_0 is the theoretical initial feed flow rate, calculated as follows:

$$F_0 = C_{X,0}V_0 \frac{\mu_{sp}}{Y_{X/S}\,S_F} \tag{5}$$

Here, $C_{X,0}$ is the initial biomass concentration, V_0 the initial volume of the culture, $Y_{X/S}$ is the biomass yield coefficient, and S_F is the substrate concentration in the feed medium. The initial biomass concentration is obtained with the dielectric measurement, while the biomass yield is determined at the end of the initial batch phase.

The feedback action of the controller is based on the process control error, calculated as follows:

$$\varepsilon(t) = \mu_{sp} - \mu_{est}(t) \tag{6}$$

The feedback controller contains proportional and integral gains (respectively, K_p and K_i). In order to avoid the use of adaptive gains, both K_p and K_i were included within the exponential term of the feedback control equation, F_{FB}, as shown in the following expression:

$$F_{FB}(t) = F_0 \exp\left(\left(K_p\varepsilon(t) + K_i \int_0^t \varepsilon(t)dt\right)t\right) \tag{7}$$

Both parts of the controller, feedforward and feedback, are used simultaneously and can be written in the same and final control equation given below:

$$F(t) = F_0 \exp\left(\left(\mu_{sp} + K_p\varepsilon(t) + K_i \int_0^t \varepsilon(t)dt\right)t\right) \tag{8}$$

This form of feedforward-feedback controller was used by Dabros et al. [24]. Its main drawbacks include sensitivity to noise and oscillatory behavior, observed to increase in time. Indeed, if the error term is unstable and noisy, the controller action can intensify the problem. To reduce the oscillations, the controller gains have to be chosen meticulously. In this work, two different methods were applied to tune the gains' values. The first approach involved manual tuning from the initial values reported in [24], which were 1.5 [-] for K_p and 0.5 h^{-1} for K_i. After several cultures, the gains were adapted according to a manual tuning methodology [45] as follows: K_p was reduced to 0.75 [-] and K_i increased to 1 h^{-1}. The second method used to calculate the controller gains was the Ziegler–Nichols open-loop tuning table [46]. This approach is based on the system's response following a step input applied to the control variable. In this work, a predefined feed flow rate was applied according to Equation (4), and the specific growth rate response was used to determine the proportional and integral terms. The calculated K_p was 0.66 [-] and K_i was 0.6 h^{-1}, both values relatively close to the ones obtained with

manual tuning. The two sets of gains were tried in order to make the final choice: 0.75 [-] and 1 h^{-1} for K_p and K_i, respectively.

By analyzing Equation (8), it can be noted that the proportional and integral terms of the controller are time dependent. This design effectively makes the weights of the two terms increase with time. Indeed, in this work, it was noticed that the oscillatory behavior increased with time not only in the control variable, μ, but also in the manipulated variable, $F(t)$. This meant that the oscillations were time dependent, and therefore the controller logic needed to be changed to remove the gains' dependence on time. To address this issue, a novel approach is proposed as follows.

First, only the proportional term was changed, yielding the following expression:

$$F(t) = F_0 \exp\left(\left(\mu_{sp} + \frac{K_p}{t}\varepsilon(t) + K_i \int_0^t \varepsilon(t)dt\right)t\right)$$

(9)

By dividing K_p by the time, the effect of this term becomes constant over time. This change should increase the performance of the controller by reducing the high-frequency oscillations in the control and manipulated variables.

Similarly, the time dependence of the integral term was suspected to induce increasing oscillations in the control and the manipulated variables. In this case, the disturbance was expected to be low in frequency, since the integral term's effect is delayed in time. Thus, by making the integral term constant, the low-frequency oscillations should be reduced. To verify this hypothesis, Equation (9) was adapted as follows:

$$F(t) = F_0 \exp\left(\mu_{sp}t + K_p\varepsilon(t) + K_i \int_0^t \varepsilon(t)dt\right)$$

(10)

This last modification was thought to be necessary particularly for long-duration fed-batch cultures. It should be noted that with the final controller logic (Equation (10)), both parts of the feedback controller have a constant weight over time, but they are still included in the exponential term of the equation. This is important because it allows for exponential gain scheduling, making the otherwise linear PI controller robust with respect to the exponential growth dynamics of the fed-batch culture.

Each time a new specific growth rate setpoint was defined during a running culture, the initial feed flow rate, F_0, was calculated anew using Equation (5), applying the latest value of the biomass concentration, $C_{X,t}$, and the current volume, V_t. To prevent integral windup, the window of integration was limited to 3 h. This limitation allowed maintaining the controller action continuous throughout the culture without having to reset the integral term between two setpoints.

Figure 2 shows the controller block diagram used in this work. The final μ controller logic is given by Equation (10). The control loop was executed every 20 s by the LabVIEW supervision program, registering all the process measurements and sending commands to the feed pump.

The performance of the controller was assessed, at each setpoint, by calculating the root mean square error (RMSE, Equation (11)). In order to allow for adequate stabilization time, the calculation window started one hour from the moment when the setpoint was initially applied and continued until the next setpoint change. The stabilization time was necessary because the dielectric signal showed signs of increased disturbance resulting from the metabolic adjustments following each setpoint change.

$$RMSE = \sqrt{\frac{\sum_i^n \left(\mu_{est_i} - \mu_{sp}\right)^2}{n}}$$
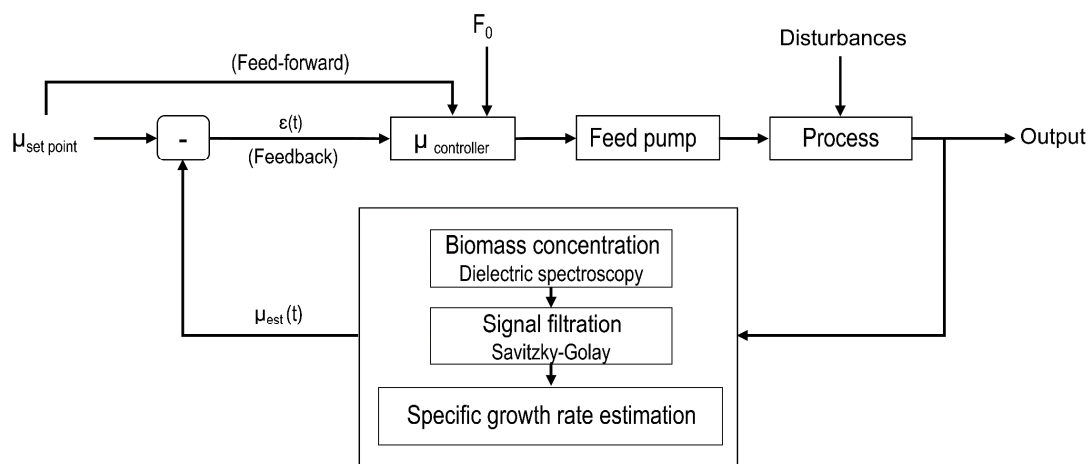
(11)

Figure 2. Controller block diagram.

3. Results

First, the performance of the original, unmodified feedforward-feedback PI controller (Equation (8), [24]) was assessed by analyzing the profiles of the specific growth rate and of the feed flow rate over the duration of a fed-batch culture. Analyzing the μ profile, shown in Figure 3, it can be observed that the process showed increasing oscillations starting after two hours of the fed-batch culture. The RMSE for this experiment was 0.063 h^{-1}, corresponding to a mean controller error of 32%.
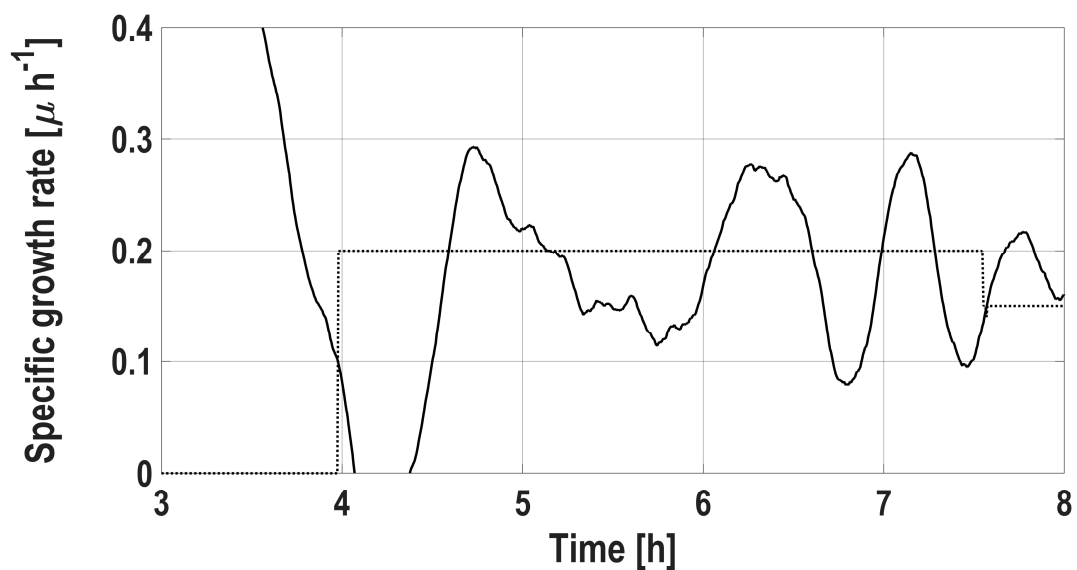
Figure 3. Setpoint (dash line) and estimated specific growth rate (solid line) during a controlled fed-batch culture, controller logic (Equation (8)).

To improve the controller and reduce the amplified oscillations, it was necessary to reduce the effect of the part of the controller action that reacts fast to the evolution of the error, $\varepsilon(t)$. Indeed, Figure 4 confirms that oscillations were equally present in the manipulated variable. This meant that the action responsible for this behavior was dictated by the proportional term.
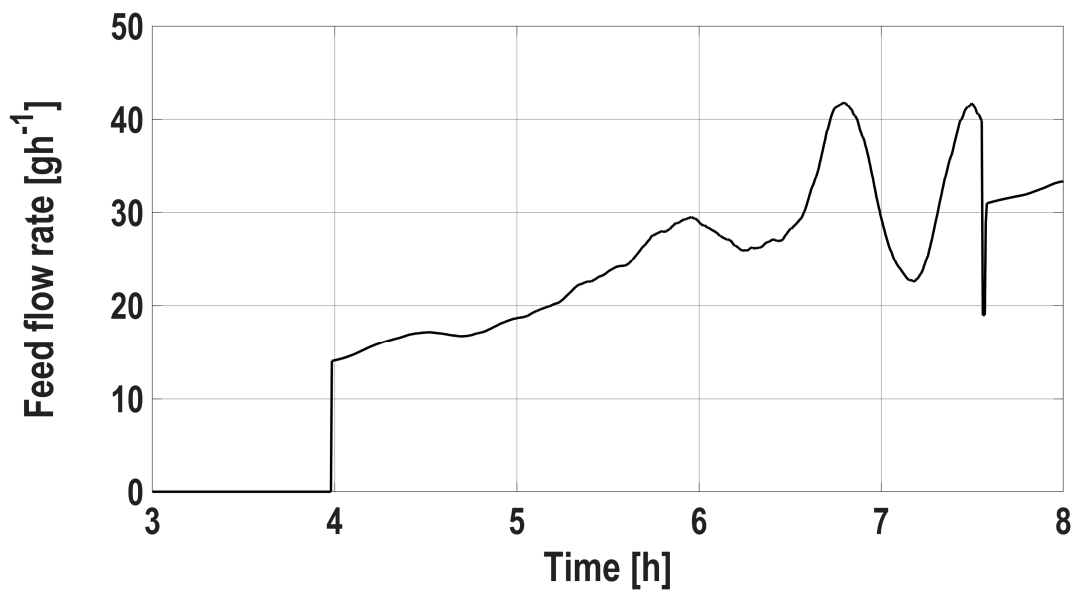
Figure 4. Feed flow rate applied during a controlled fed-batch culture, controller logic (Equation (8)).

For the subsequent experiment, the controller logic was changed to the one given by Equation (9). By maintaining the proportional gain, K_p, constant overtime, the high-frequency oscillatory behavior was reduced. In order to assess the controller's improvement, a longer step was performed. Figure 5 shows the specific growth rate profile over a nine-hour duration fed-batch culture. The process still shows oscillations, but ones that are constant and not amplified over time. In this experiment, the root mean square error (RMSE) was 0.072 h^{-1} which corresponded to a mean controller error of 36%. It was stipulated that the controller still could be improved by examining the corresponding feed flow rate profile, shown in Figure 6. The oscillatory behavior was still present in this signal, but its frequency was lower (~0.5 h^{-1}) than that observed previously (Figure 3, ~1 h^{-1}). Nevertheless, the amplitude of the oscillations did increase in time.
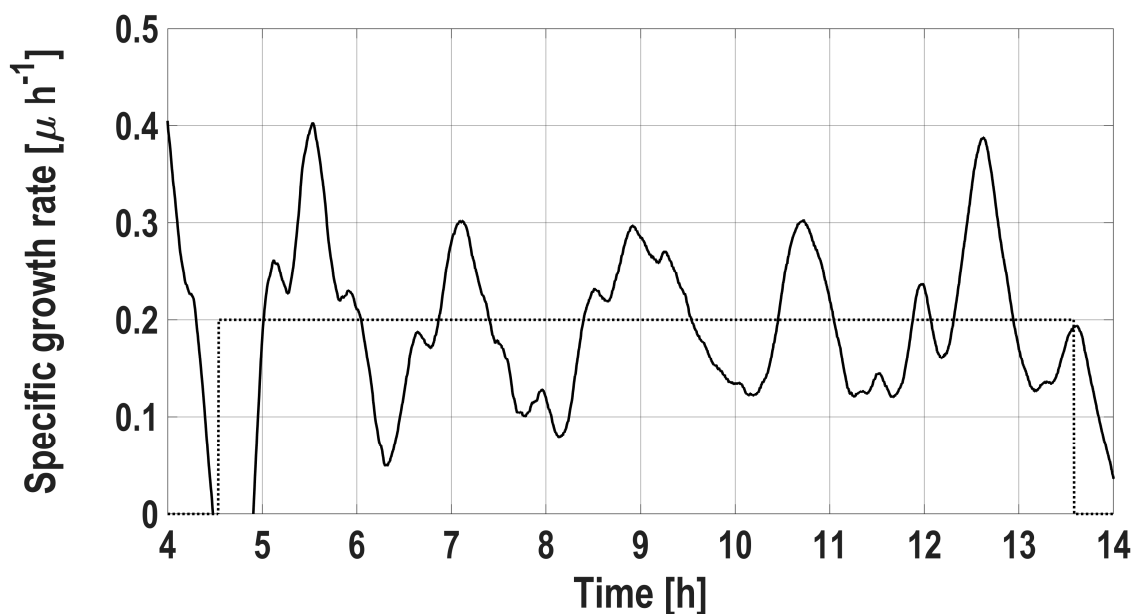

Figure 5. Setpoint (dash line) and estimated specific growth rate (solid line) during a controlled fed-batch culture, controller logic (Equation (9)).
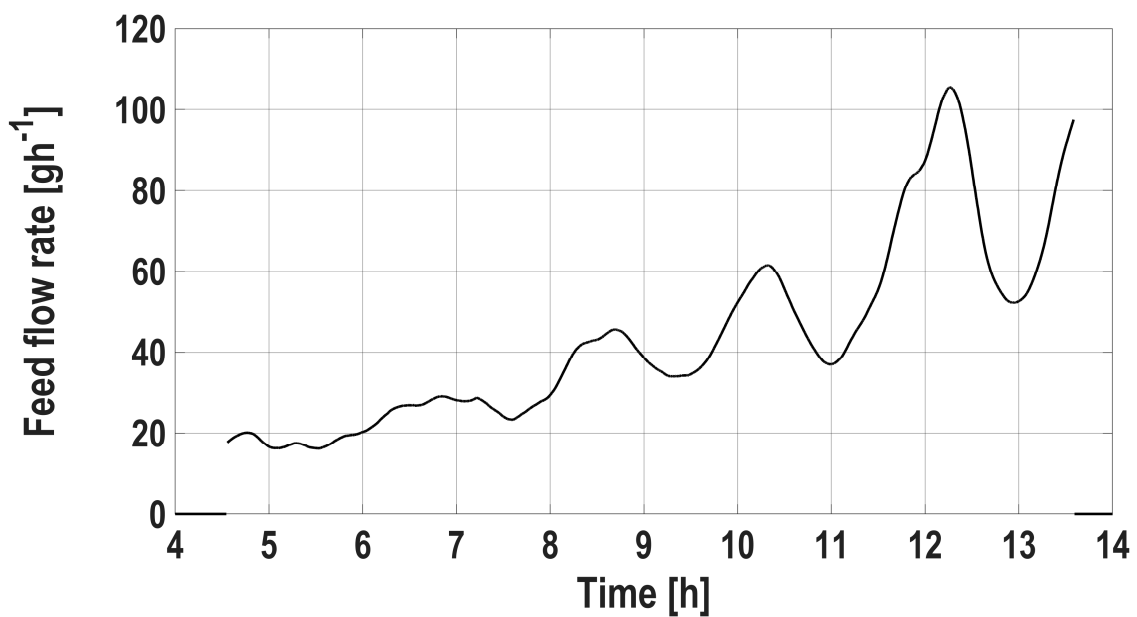
Figure 6. Feed flow rate profile obtained during a controlled fed-batch culture, controller logic (Equation (9)).

As the proportional gain remained constant, it could not be responsible for amplifying the oscillations in the feed rate. On the other hand, the integral term was still time dependent, its effect increasing over time. Moreover, since the oscillatory frequency was lower, suggesting that the term responsible for inducing them was a slow-acting term, it pointed at the integral term. Following this consideration, the controller logic was changed to that given in Equation (10).

To assess the improvement in the controller's performance, another experiment was performed where the specific growth rate setpoint was changed mid-culture. With the theoretical maximal specific growth rate of *K. marxianus* being close to 0.6 h^{-1}, it was decided to control the growth rate at a high and a low setpoint within the same culture [40,47,48].

Figure 7 shows the specific growth rate obtained during this fed-batch experiment. It was possible to control the growth rate at 0.4 h^{-1}; however, the culture could not be run at that setpoint for a long time. Indeed, the cell concentration increased too rapidly to allow for full aerobic conditions (DO > 20%). After 3 h, the limit of 20% DO was reached, and the setpoint was lowered to maintain the culture in aerobic respiration. It is unclear whether the remaining mild oscillatory behavior was caused by the residual noise in the filtered dielectric signal or by the controller itself. Figure 8 shows the feed flow rate applied throughout the culture; this time, little oscillation was observed in the manipulated variable. The resulting smooth control action allowed the controller to reach and maintain the growth very close to the setpoint throughout the experiment. The root mean square error (RMSE) for the μ setpoint of 0.4 h^{-1} was 0.061 h^{-1} and for the setpoint of 0.1 h^{-1}, the RMSE was 0.019 h^{-1}. The combined mean controller error for this culture was 17% which was the half compared to the errors observed during the cultures where the controllers given by Equations (8) and (9) were used (Figures 3 and 5, combined mean controller error of 32% and 36%, respectively).
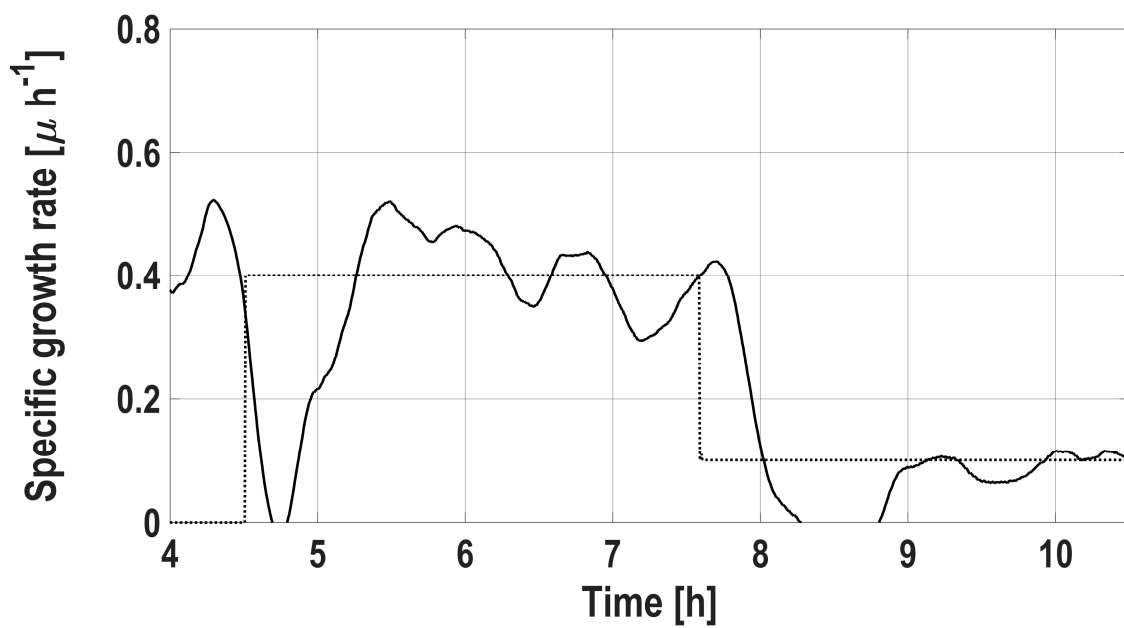
Figure 7. Setpoint (dash line) and estimated specific growth rate (solid line) during a controlled fed-batch culture, controller logic (Equation (10)).
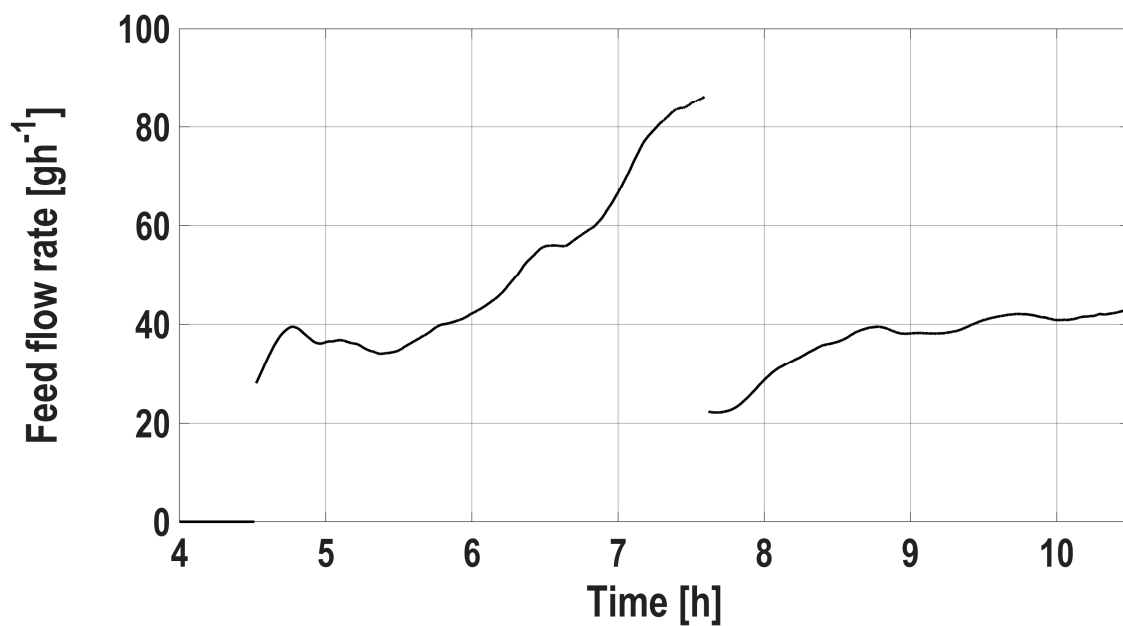

Figure 8. Feed flow rate profile during a controlled fed-batch culture, controller logic (Equation (10)).

Figure 9 shows the evolution of the biomass concentration during this fed-batch culture. The individual stages of the culture can easily be identified, i.e., the initial batch phase (until culture time of 4.4 h), followed by the two fed-batch phases at μ setpoints of 0.4 h^{-1} and 0.1 h^{-1}.
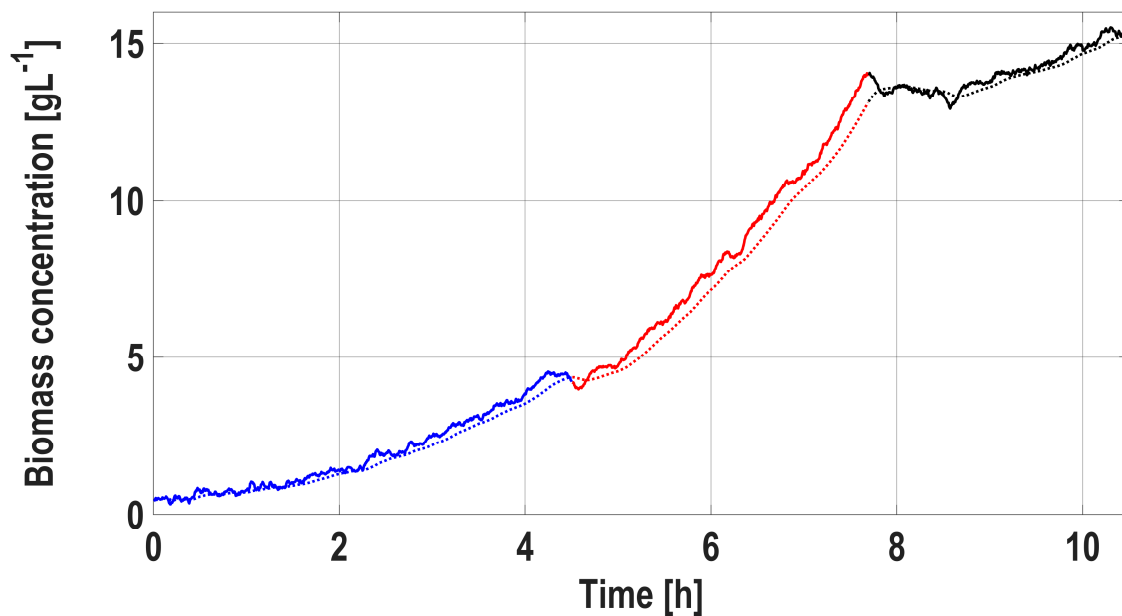
Figure 9. Evolution of biomass concentration (raw signal in solid line, filtered signal in dashed line) during the initial batch phase (blue) and two fed-batch phases at μ_{sp} = 0.4 h^{-1} (red) and 0.1 h^{-1} (black).

4. Discussion

In this work, it was demonstrated that the logic of the specific growth rate controller was equally, if not more, important than the controller gains. Indeed, the control performance changed significantly depending on the approach chosen.

As a first step in the study, we have addressed the weakest element in the control loop, namely, the biomass measurement and the specific growth rate estimation. The biomass measurement signal was inherently noisy and sensitive to process conditions, such as aeration and agitation. The filtering approach was optimized to minimize the effect of the bubbles without inducing too long a lag time. The window of 15 min chosen for the specific growth rate estimator was a trade-off with respect to the lag time of the filtering algorithm and the remaining noise on the biomass signal.

Secondly, it was illustrated that by adjusting the controller logic, the signal oscillations could be managed effectively, and the controller stability improved. Using the original controller (Equation (8)), a fed-batch culture lasting longer than 2 h was difficult to achieve because of increasing oscillations in both the control and the manipulated variables. With the new control logic, the experiment was shown to be stable in time, and the mean controller error was reduced to 17%. The remaining error seems to be limited by the residual noise amplitude on the biomass measurement.

Finally, the reproducibility of the controller's performance was assessed by comparing three fed-batch cultures run at the same specific growth setpoint (0.1 h^{-1}). The result, shown in Figure 10, demonstrates that after the initial period of instability (1 h), the estimated μ tracked the assigned setpoint in all three runs. The level of accuracy was similar, showing an average relative RMSE of 23% ± 6% (1σ) despite the variable starting biomass concentration, indicating controller robustness.

All fed-batch cultures were run between 6 and 9 h in total duration, with variable μ setpoints. Throughout this work, the maximum biomass concentration allowing aerobic conditions was around 25 g/L of dry cell weight. Above this concentration, regardless of the specific growth rate setpoint, the oxygen transfer rate was insufficient to allow purely aerobic respiration of the cells. The aim of fed-batch cultures is to ensure a permanent state of substrate limitation. If the culture conditions changed from aerobic to anaerobic, the limiting factor would be a combination of substrate and oxygen. The agitation rate could be increased to enhance the oxygen transfer and allow for higher biomass concentrations. However, this change was observed to deteriorate the growth rate estimation by increasing significantly the noise in the biomass concentration measurement.
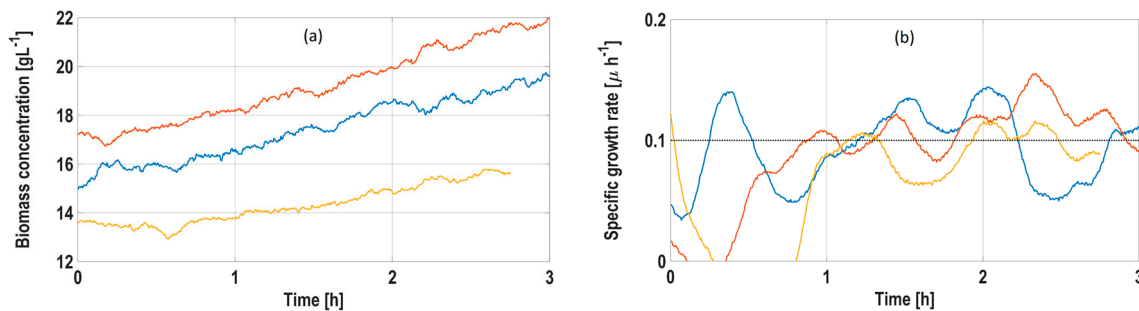
Figure 10. Biomass concentration profiles (**a**) and specific growth rate estimates (**b**) during three fed-batch phases run at $\mu_{sp} = 0.1 \text{ h}^{-1}$.

5. Conclusions

The aim of this work was to implement and optimize a novel proportional-integral (PI) feedforward-feedback controller, designed to maintain a desired specific growth rate of a microbial culture. The proposed new control logic provides robust setpoint tracking of an exponentially evolving fed-batch culture, while ensuring improved noise and oscillation management. To reduce the noise present in the biomass measurement, the filtering approach was optimized using a first-order Savitzky–Golay algorithm instead of a simple moving average. Then, to limit the oscillations in the manipulated and control variables, the time dependency of the P and I feedback terms was removed, as shown in Equation (10). This change enhanced the stability of the system for longer processes without any noticeable increase in signal oscillations, as was previously observed. Despite the remaining noise in the biomass measurement, the controller was able to cope with this issue and maintain the desired specific growth rate over the duration of the culture. In conclusion, it was shown that a strain of *K. marxianus* could be grown successfully in fed-batch mode, under substrate-limited, aerobic conditions, at different setpoints ranging from 0.1 h^{-1} to 0.4 h^{-1}.

Some aspects could still be improved, particularly the biomass measurement signal filtration. In order to diminish the undesired effect of aeration on the dielectric signal, two possibilities become apparent. The first solution is to work under a slight pressure in the reactor, which would increase the solubility of oxygen, and thereby reach aerobic conditions with a reduced aeration rate. The second solution is to use an external bypass (flow-through cell) where the probe could be installed to measure the medium permittivity without the presence of bubbles. The latter approach would require modifications to the existing bioreactor. Finally, a more powerful signal filtration technique could be applied, such as the Kalman filter [22].

It was noticed that the biomass yield varied between the fed-batch cultures that were performed. Depending on the specific growth rate, the obtained biomass yield, $Y_{X/S}$, varied from 0.3 to 0.65 g/g. This fact complicated the use of the initial feed flow rate calculation, (F_0, Equation (5)) since both terms, μ_{sp} and $Y_{X/S}$, are present in this equation and yet, they are interdependent. It could be interesting to investigate more in depth the effect of the specific growth rate on the biomass yield, as suggested in [5]. By knowing at which growth rate a particular strain is more productive, industrial applications could be optimized and their costs reduced.

This work highlighted the feasibility of controlling the specific growth rate of yeast in long-duration fed-batch cultures and in spite of a noisy biomass measurement signal. The proposed approach should be applicable to other microbial systems, as well as to mammalian cell cultures. In the latter case, it is expected that the slower growth dynamics should allow a further reduction in signal noise and oscillations.

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