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Fuzzy Logic-Based Adaptive Control of Specific Growth Rate in Fed-Batch Biotechnological Processes. A Simulation Study

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Abstract: This article presents the development and application of a distinct adaptive control algorithm that is based on fuzzy logic and was used to control the specific growth rate (SGR) in a fed-batch biotechnological process. The developed control algorithm was compared with two adaptive control systems that were based on a model-free adaptive technique and gain scheduling technique. A typical mathematical model of recombinant *Escherichia coli* fed-batch cultivation process was selected to evaluate the performance of the fuzzy-based control algorithm. The investigated control techniques performed similarly when considering the whole process duration. The adaptive PI controller with fuzzy-based parameter adaptation demonstrated advantages over the previously mentioned algorithms—especially when compensating the deviations of the SGR. These deviations usually occur when the equipment malfunctions or process disturbances take place. The fuzzy-based control system was stable within the investigated ranges. It was determined that, regarding control quality, the investigated control algorithms are suited to control the SGR in a fed-batch biotechnological process. However, substrate feeding rate manipulation and limitation needs to be used. Taking into account the time needed to design and tune the controller, the developed controller is suitable for practical applications when expert knowledge is available. The proposed algorithm can be further adapted and developed to control the SGR in other cell cultivations while running the process under substrate limitation conditions.

Keywords: biotechnological process; adaptive control; specific growth rate control; fuzzy logic

1. Introduction

The modern food, chemical and pharmaceutical industry go hand in hand with biotechnology. The production of various recombinant proteins is a big part of the sourcing of pharmaceutical ingredients. These processes can be described as nonlinear and nonstationary, making modeling and control a complicated control engineering task. In the biotechnology industry, this is even more challenging due to strict safety regulations and operational constraints [1–3].

One of the most important control engineering tasks is the development of straightforward and robust methods that could be used to monitor and control the specific growth rate (SGR) in industrial bioreactors. This is often needed to successfully implement a Process Analytical Technology (PAT) framework in bioengineering [4]. Nevertheless, currently, mostly relatively basic control systems are used in most industrial-scale bioreactors, even though advanced control strategies are extensively discussed in the academic community [5].

The biomass SGR, which can be described as the ratio between the biomass absolute growth rate and the biomass amount accumulated in the culture broth, can be considered one of the most important

variables in biotechnological processes. Not only does SGR influence the physiological state of a microbial culture, it also defines the production of desired products, their quantity, and quality [3,6,7], and also the rate of product synthesis. Effects like substrate inhibition or overflow are fairly common in fed-batch bioreactors. However, these effects can be dealt with if controlled properly. A well set-up control system is able to yield high product concentrations as well as high cell densities. This can be reached by maintaining the substrate concentration at certain levels leading to a controlled biomass growth rate [1] that can be achieved by changing the substrate feeding rate.

In many cases, biotechnological processes are controlled using PID (proportional–integral–derivative) controllers, which usually operate in basic control systems. Temperature, pH and other simple process variables are usually controlled in these systems. Control quality gravely relies on the development and tuning of the controller and its ability to deal with process variability and disturbances [1,2,4]. Common PID controllers that use constant tuning parameters are unable to achieve the required control accuracy of the process since the dynamics significantly vary during the operation. The academic community has proposed various PID controller parameter tuning approaches that take into account time-varying operating conditions: rule-based fuzzy systems [8], first-principle models [9], gain scheduling methods [10–12], and other techniques [1,2,13–15]. For exponentially evolving processes, a technique based on feedforward-feedback control was proposed [16]. The proposed methods provide a basis for both theory and practice enabling the utilization of adaptive control algorithms in biotechnological process control and highlight that the application of these control methods can add significant value to the performance of basic control systems.

In this work, a fuzzy-based adaptive control algorithm is developed and tested. The performance of the presented controller is then compared with other adaptive control systems that are based on a model-free adaptive technique and gain scheduling technique. These algorithms were applied to control biomass specific growth rate in a typical production process of a bioreactor-scale fed-batch recombinant protein. A mathematical model of the process was used to carry out numerical simulations. The control system's feedback signal was sampled using realistic intervals. The data were also corrupted by additive noise during the simulation. During the simulation runs the performance of the system was also evaluated. An impartial comparison is thus possible due to the fact that unknown experimental or biological variability is avoided. Nevertheless, the extensive additional experimental investigations are planned to test the control system performance under real conditions.

2. Materials and Methods

2.1. Mathematical Model of the Biotechnological Process

In this study, the biotechnological process was simulated using the mathematical model of the *Escherichia coli* BL21 strain. This strain also harbored a pBR322 plasmid derivative and was cultivated in a recombinant fed-batch process. Mainly because this recombinant protein is used in typical bioreactor-scale processes.

The cultivation of the selected recombinant protein can be described as a two-phase process. The target of the first phase is to accumulate the bioreactor with a sufficient biomass. The specific growth rate is usually relatively high for this phase. During phase two, the recombinant protein is produced. During the described two phases, different optimal temperatures were defined and maintained for each phase. In the simulated process, the broth temperature was maintained at 37 °C during the first phase thus maximizing the biomass growth to an optimal level. At the start of the production process, the temperature was reduced to 32 °C. The substrate feeding rate profiles and the induction time also have a significant influence on the process performance. Therefore, they are also subject to model-based optimization. The induction time (8 h) for the investigated process of a given total duration was determined in [17] using model-based optimization techniques. During the model-based optimization of the process [17], the process performance (productivity) index equal to the total target protein amount at the end of the process was used. The off-line measurement techniques applied for biomass and target

protein analysis in the investigated process are described in detail in [17]. For the online measurements of the biomass concentrations, alternative techniques based on turbidity (optical density), permittivity measurements or off-gas analysis can be used. For protein measurement, a promising alternative to the applied techniques are model-based approaches (application of soft-sensors, generic estimators, e.g., [18]) that would enable the online monitoring of the key process variables.

A mathematical model presented in [17] was used to simulate the described biotechnological process. According to other studies, pre-optimized feeding rate profiles are used to control the biomass growth and protein production [17]. This type of open-loop system can be controlled effectively only then when no considerable process condition deviations or equipment disturbances/malfunctions take place. Failure to do so may lead to deviations in the specific growth rate thus reducing the process productivity. Therefore, the SGR was selected as the main controlled variable of this research that would be maintained by controlling the substrate feeding rate. In this study, an adaptive closed-loop control algorithm was used for keeping the optimal SGRs trajectory on track. The discussed process can be described using the bellow provided differential equations.

$$\frac{dx}{dt} = \mu(s, T)x - u \frac{x}{w} \quad (1)$$

$$\frac{ds}{dt} = -q_s(s, T)x + u \frac{s_f - s}{w} \quad (2)$$

$$\frac{dp_x}{dt} = q_{px}(\mu, p_x) \quad (3)$$

$$\frac{dw}{dt} = u + F_{smp} \quad (4)$$

where x (g/kg) describes the biomass concentration. μ (1/h) is the biomass specific growth rate that is a function of the glucose concentrations (g/kg) and culture broth temperature T ($^{\circ}\text{C}$). Here, w (kg) is the culture broth weight, q_s (g/(gh)) is the glucose specific consumption rate. The glucose concentration in the feeding solution is noted as s_f (g/kg) while p_x (U/(g biomass)) is the specific protein activity. q_{px} (U/(gh)) is the specific protein accumulation rate; u (kg/h) and F_{smp} (kg/h) are the substrate feeding and the sampling rates, respectively.

SGR is modeled using the Haldane-type model [19]:

$$\mu(s, T) = \mu_{max} \frac{s}{K_s + s} \frac{K_i}{K_i + s} \exp(\alpha(T - T_{ref})) \quad (5)$$

where μ_{max} (1/h) is a maximal specific growth rate parameter. K_i and K_s (g/kg) are then inhibition and Monod constants. How the temperature influences the growth rate is considered with the parameter α ($1/^{\circ}\text{C}$). The optimal growth phase temperature is noted as T_{ref} ($^{\circ}\text{C}$). The optimal temperatures that yield the highest growth and protein synthesis rates were determined in the previous study [20].

The specific consumption rate of the substrate q_s is given by the following expression:

$$q_s(s, T) = \frac{1}{Y_{xs}} \mu(s, T) + m \quad (6)$$

where m (g/(gh)) is a maintenance term and Y_{xs} (g/g) is a conversion yield coefficient. This consumption rate is proportional to the cell growth and rate of substrate consumption. The vital functions of the cell are also considered by implementing a maintenance term.

According to research on various recombinant proteins as target products, the SGR and the temperature are stated as the essential parameters that influence the protein production rate [19]. However, it could be that both parameters, depending on the particular target product, may differ. Inclusion body formation and soluble protein formation could be taken as an example, since the first requires a relatively high value of the SGR compared to the second process [20–25]. In the simulation,

the mathematical model of the target accumulation rate of the product q_{px} considers the actual protein activity and the influence of the SGR:

$$q_{px}(\mu, p_x) = \frac{1}{T_{px}}(p_{max}(\mu) - p_x) \quad (7)$$

$$p_{max}(\mu) = \frac{\mu K_m}{K_\mu + \mu + \mu^2 / K_{i\mu}} \quad (8)$$

where T_{px} (h) is a protein accumulation time constant. The maximal specific protein activity is noted as $p_{max}(\mu)$ (U/(g biomass)). This parameter also depends on the SGR: K_m (U/(g biomass)), K_μ (1/h) and $K_{i\mu}$ (1/h) are Monod and inhibition constants, respectively. The oxygen uptake rate OUR (g/h) was described using the Luedeking–Piret-type model [25,26]:

$$OUR = (Y_{ox}\mu + m_{ox})xw \quad (9)$$

where Y_{ox} (g/g) is a coefficient describing the conversion yield and m_{ox} (g/(gh)) is a maintenance term.

Equations (1)–(9) were used for modeling the described fed-batch process' behavior and to analyze the proposed adaptive closed-loop control algorithm's control performance. In this research it is presumed that the bioreactor is ideally mixed and neither actuators nor measurement devices cause significant time delays that may influence the control quality. The identified model parameter values used in this simulation can be found elsewhere [19].

2.2. Adaptive Control Algorithm

Considering the implementation of an SGR control algorithm in an industrial biotechnological process, it should be user-friendly and relatively simple, subject to standard control and measurement equipment, easy to tune and to develop. Adaptation properties that consider the parameter change over time during the process should also be considered in these type of control algorithms. Based on the above considerations, a PI controller with fuzzy-based parameter adaptation was developed and investigated.

The application of an ordinary PID controller is limited due to the nonstationary and nonlinear behavior of the described biotechnological processes. An adaptation of the controller parameters may be needed because of the dynamic response variation considering the systems operation point. To fulfill the control quality and stability requirements, an adaptation of the controller parameters is needed. This can be realized by using adaptive control techniques. Therefore, a fuzzy model was designed and implemented to adapt the PI controller parameters in real time. As the SGR cannot be directly measured online, a state estimator and an auxiliary variable for the calculation of the signal were used [19]. These variables are then used for the feedback loop and fuzzy model input. In this study, it was decided that the OUR would be a suitable additional variable due to the fact it not only reflects the cultures physiological state but has a good correlation with the SGR. Nevertheless, it is possible to accurately measure it during a bioreactor-scale cultivation process. The obtained results show that the biomass concentrations calculated from the estimated SGR fit well to the measured (reference) biomass concentrations. Figure 1 represents the general structure of the developed fuzzy-based PI controller.

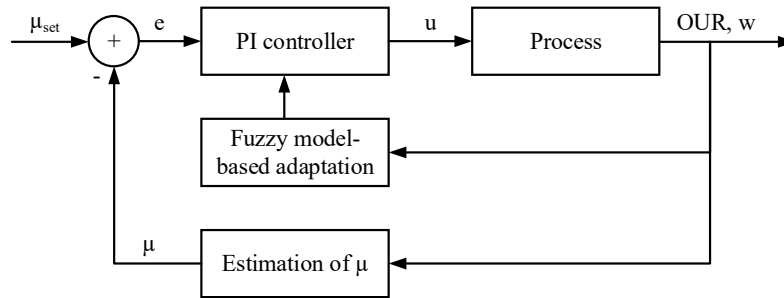


Figure 1. General structure of the fuzzy-based adaptive PI control system for specific growth rate control.

2.2.1. Fuzzy-Based Algorithm Design

To create a fuzzy-based adaptive PI controller, input and output variables for the fuzzy system need to be defined. Since the function of the fuzzy model is to adapt the PI controller parameters, controller gain K_c and integration time constant T_i parameters were selected as the output variables. Both T_i and K_c depend on the process variables as described in [19]:

$$K_c \propto k_0 w \tag{10}$$

$$T_i \propto \frac{k_1}{OUR/w + k_2} \tag{11}$$

where k_0 , k_1 and k_2 are fixed parameters, the values of which are subject to optimization and were calculated and discussed in detail in [19]. The aim of the optimization was to find a set of the parameters that minimizes the tracking error (e.g., integral time absolute error (ITAE)) of the designed control system when using the Equations (10) and (11) for controller parameter adaptation. Since the PI controller parameters mainly depend on OUR and w , both process variables need to be used in the fuzzy system. To decrease the complexity of the fuzzy system, the ratio of these two process variables was used as the input for the fuzzy system. According to the heuristic knowledge available, the following rules were created for the fuzzy system:

- IF OUR/w is poor, THEN T_i is big
- IF OUR/w is excellent, THEN T_i is small
- IF OUR/w is poor, THEN K_c is small
- IF OUR/w is excellent, THEN K_c is big

Both for the input and output of the fuzzy model, two Gaussian membership functions were selected. To avoid the extensive overlapping of the membership functions, the number of used membership functions per parameter was decreased to two (see Figure 2).

Whether a fuzzy application is implemented successfully depends on a number of factors. One of these are the membership functions of the fuzzy model. However, these are usually decided upon subjectively. One way to improve the performance of the fuzzy model is the use of genetic algorithm (GA). Various studies have shown that GAs and other heuristic algorithms are able to improve the systems performance by optimizing the membership functions [27–29]. Due to its simplicity and straightforwardness, a genetic algorithm [30] was selected to find the optimal membership function parameters. GAs adapt a direct analogy of natural evolution to perform global optimization in order to solve highly complex problems. It assumes that the possible problem solution is individual and can be described by a set of parameters. These parameters are coded as genes of a chromosome and can be structured by a string of concatenated values. Variable representation is defined by the encoding scheme that can be represented by various forms like binary or real numbers, depending on the used data. The search space of the data is usually defined by the problem. In this research, the fuzzy model membership function parameters were coded in the chromosomes. At the beginning, an initial

chromosome is randomly generated. Then, the fitness values of all chromosomes are evaluated by calculating the objective function in a decoded form. The integral time absolute error (ITAE) value of the SGR setpoint tracking was selected as the fitness function since this criterion was also used for the systems performance evaluation. Determined by the fitness of each individual, the best chromosomes are grouped, based on the lowest ITAE value, and the group with the lowest value is then selected in the selection process. The genetic operators like crossover and mutation are applied to this selected population in order to improve the next generation solution. The process is repeated until the population converges to the global minimum or another termination criterion is reached. In the reproduction phase, the fitness value of each chromosome is evaluated, and it is used in the selection process to provide bias towards fitness individuals. Then, a crossover algorithm is initiated once the selection process is completed. The background operator in genetic algorithms is mutation. The parameters of the GA directly depend on the number of variables. In the recommendations provided by the authors of the GA, the number of generations should be equal to $N_{var} * 20 + 10$, and the population number is calculated as $N_{var} * 50$ by default. After manual tuning of the membership function parameters based on heuristic knowledge of the process, 10 membership function parameters were selected for further optimization with the GA, thus simulating 210 generations with 500 populations. The large number of generations did not bring significant changes, so it was decided to use 50 generations for testing. The mutation and crossover parameters were left at their default values. In Table 1, the parameters used for the GA are presented.

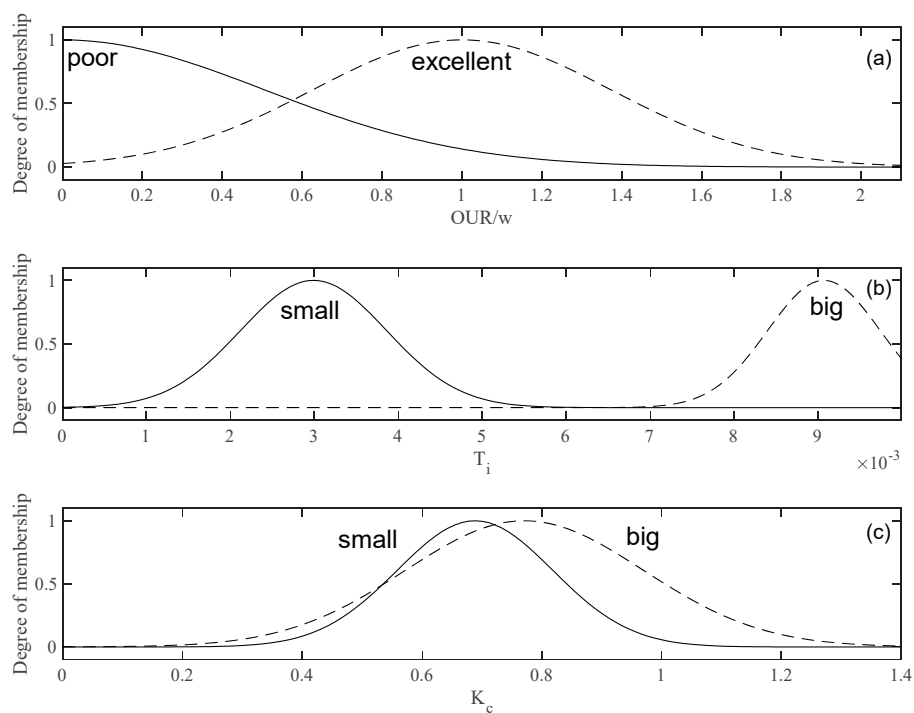


Figure 2. Fuzzy model membership functions: model input membership functions (a), model output T_i membership functions (b), model output K_c membership functions (c).

Table 1. Genetic algorithm parameters.

Number of Generations	Individuals in One Generation	Mutation Probability	Crossover Probability
50	500	0.1	0.9

This GA was used to search for the optimal parameters of the fuzzy controller membership functions. Each individual represents a simulation of the biotechnological process where the PI controller parameters are adapted using the generated fuzzy model. The structure of the controller is

constant and does not change during the simulation of the process. The GA searches for the optimal fuzzy model structure by defining the membership function parameters. In the applied genetic algorithm, the only termination criterion was the predefined number of generations. This allowed avoiding early convergence and local optima. After 50 iterations the GA was stopped as shown in Figure 3.

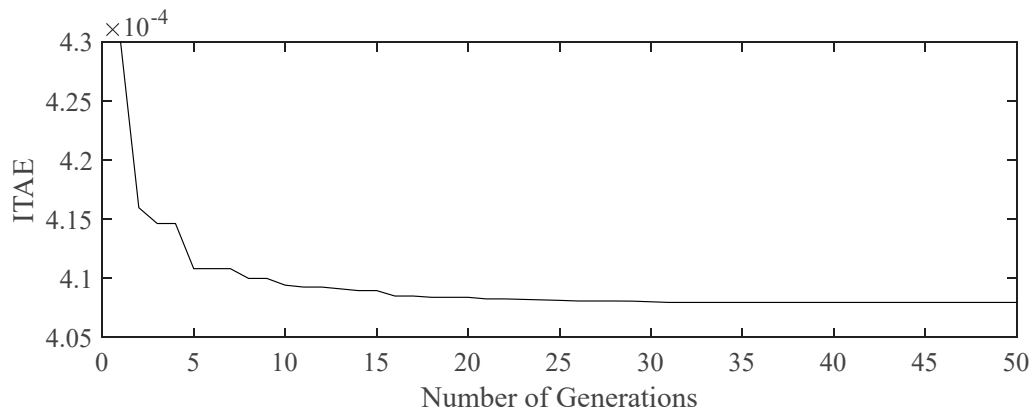


Figure 3. Integral time absolute error (ITAE) criterion change during each generation of the genetic algorithm.

The developed PI controller with fuzzy-based adaptation was implemented in the model simulation and used for the performance evaluation of the system. The K_c and T_i dependencies are presented in Figure 4.

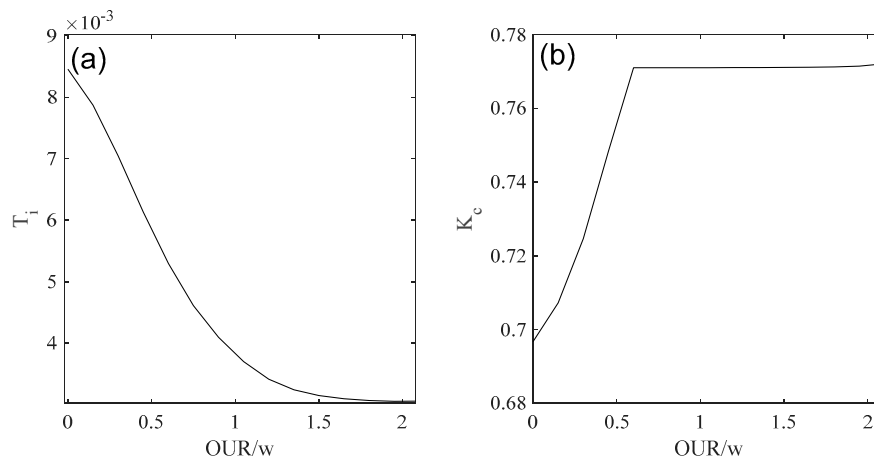


Figure 4. PI controller parameter dependencies generated by the fuzzy model: integration time constant T_i (a), controller gain K_c (b).

2.2.2. Performance Evaluation

A typical pattern of several setpoints of different length and amplitude also including disturbances of the specific growth rate were selected and modeled to evaluate the behavior of the designed control model. An example of the setpoint profile of the SGR in a simulated process run is shown in Figure 5. The control quality was tested in various operation points of the process by changing the SGR setpoint every hour from 3 to 8 h. These switching points are characterized by different SGR setpoints and accumulated biomass concentrations. Additionally, in order to evaluate the controller’s ability to remain stable and compensate disturbances at various process phases, short pump faults were simulated at 4.5, 7.5 and 9 process hours.

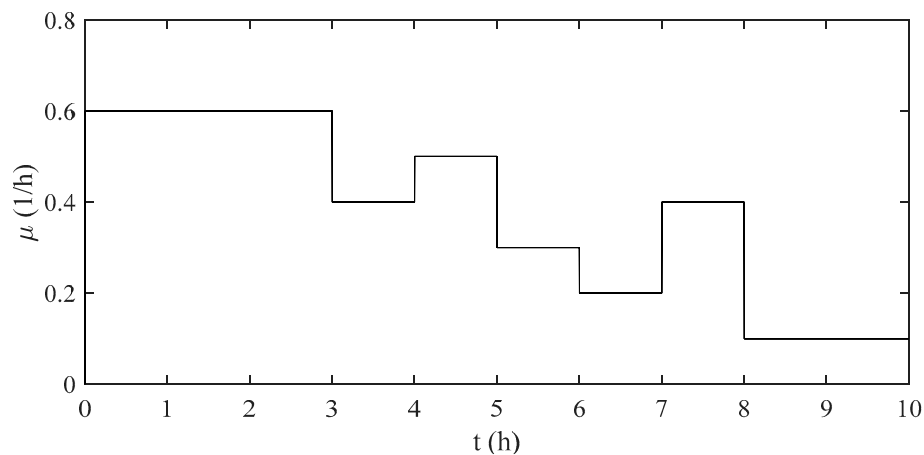


Figure 5. Selected specific growth rate setpoint profile for the biotechnological process simulation.

The ITAE criterion was selected to evaluate and compare the performance of the developed control algorithm. The adaptive PI controller with fuzzy-based parameter adaptation was compared with the gain scheduling (GS) and model-free adaptive (MFA) algorithms investigated in [19] using the same mathematical model of the biotechnological process (Equations (1)–(9)). The GS method uses additional or already existing online measurements to adapt the controller parameters using the derived functional relationships. The identification of such relationships requires an in-depth analysis of the dynamic properties of the controlled process. The MFA control technique is an alternative data-based approach that does not require deep process knowledge needed for the creation of the process mathematical model. A two-layer neural network with an input layer that has a time-delayed sequence of the tracking errors [19] was used for comparison.

3. Results

An example of the control performance of the developed PI controller with fuzzy-based parameter adaptation is presented in Figure 6. The system remains stable in a wide setpoint range. Additive white Gaussian noise was used to corrupt the OUR signal during the simulations.

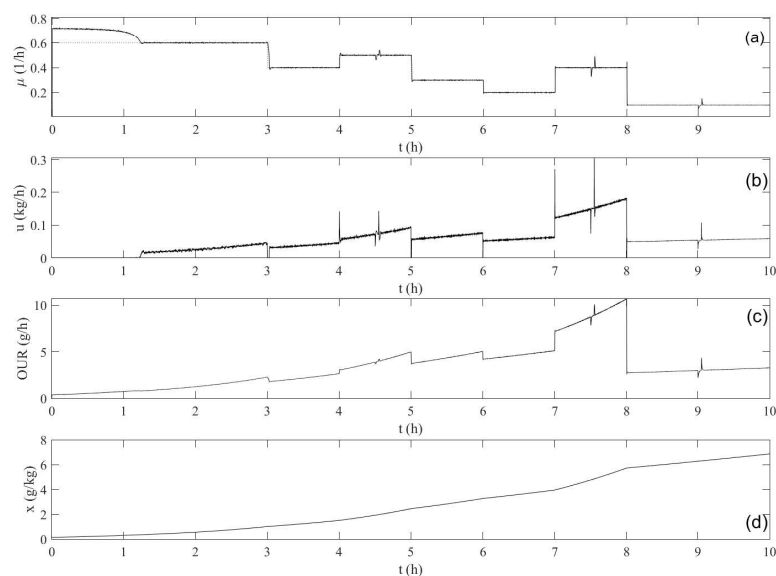


Figure 6. Specific growth rate (SGR) (system output variable) (a), substrate feeding rate (control variable) (b), oxygen uptake rate (OUR) (c) and biomass concentration (d) trajectories during the simulated process.

The change over time of the corresponding PI controller tuning parameter is presented in Figure 7. The controller gain parameter K_c (see Figure 7a) changes over time by approx. 10% only due to the fact that the parameter correlates with the culture broth weight w that increased only slightly during the simulated process. The integration time constant T_i (see Figure 7b) follows the changes of the *OUR* (see Figure 6c) profile and, therefore, reflects the significantly varying dynamics of the process.

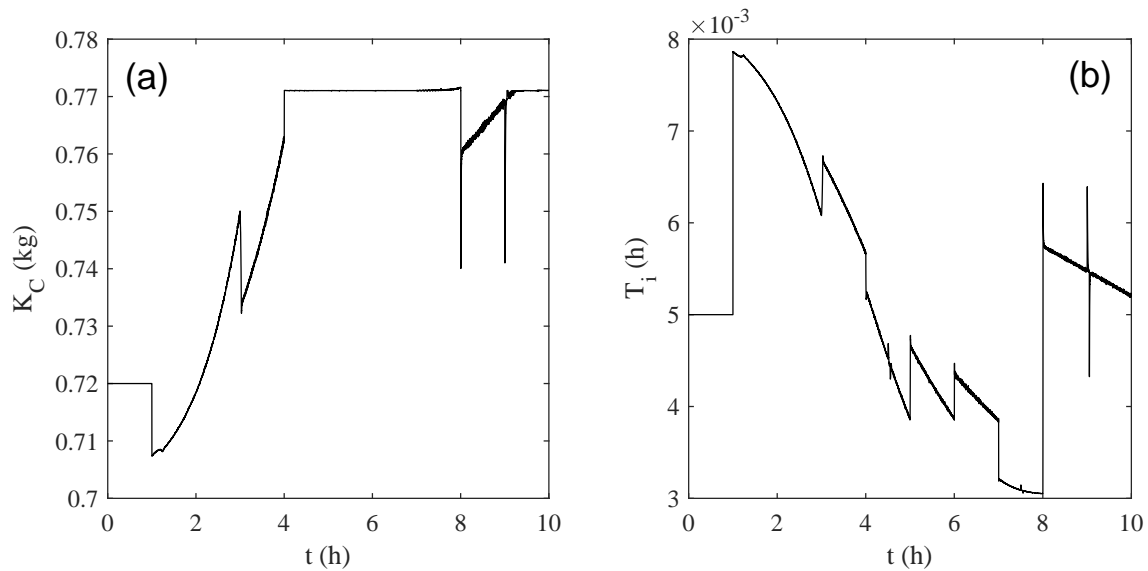


Figure 7. Development of the PI controller tuning parameters during the simulated process, (a) the controller gain parameter K_c , (b) the integration time constant T_i .

The performance evaluation summary of all the methods can be found in Table 2. Here one can see that the PI controller with fuzzy-based adaptation was able to perform similar to the gain scheduling PI and MFA control algorithms in the investigated process with a given setpoint profile and acting disturbances.

Table 2. Performance results of the investigated control algorithms.

Control Type	ITAE		
	MFA Adaptation	GS Adaptation	Fuzzy Adaptation
Setpoint-tracking	0.6865×10^{-3}	0.6592×10^{-3}	0.6723×10^{-3}
Disturbance rejection	0.3783×10^{-3}	0.3962×10^{-3}	0.3431×10^{-3}
Setpoint-tracking and disturbance rejection	0.7598×10^{-3}	0.7357×10^{-3}	0.7349×10^{-3}

All methods performed very similarly during setpoint tracking, while the fuzzy-based adaptation model performed best during disturbance rejection when comparing with the other two models. The MFA model tends to perform better in phase II of the cultivation process as shown in Figure 8.

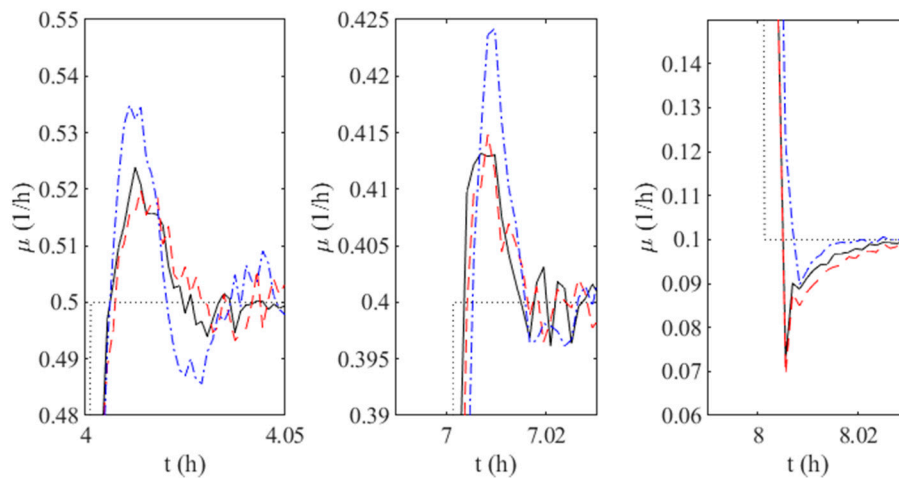


Figure 8. Transient processes of the SGR in the simulated process. Setpoint change: PI controller with fuzzy-based adaptation (– solid line), PI controller with gain scheduling (– dashed line), model-free adaptive (MFA) (-·- dash-dot line).

When handling disturbances in the system, the PI controller with fuzzy-based parameter adaptation is able to outperform the GS and MFA algorithms in the first phase of the process; however, the MFA algorithm is again better suited for the second phase of the process as seen in Figure 9.

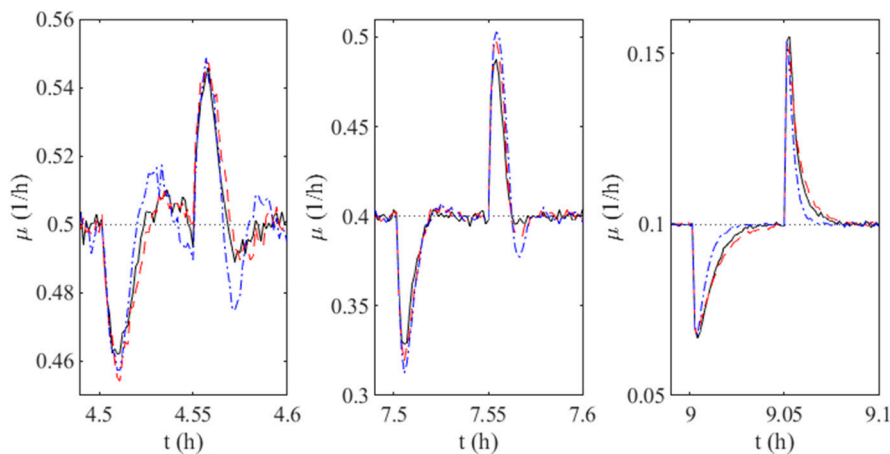


Figure 9. Transient processes of the specific growth rate during a simulation run. Disturbance rejection when using: PI controller with fuzzy-based adaptation (– solid line), PI controller with gain scheduling (– dashed line), MFA (-·- dash-dot line).

4. Discussion

In general, the investigated control techniques perform similarly when considering the whole process duration. The type of controller that should be selected, therefore, depends on the existing knowledge of the process and the control type. The adaptive PI controller with fuzzy-based parameter adaptation demonstrates advantages over the gain scheduling PI and model-free adaptive algorithms when compensating the SGR deviations that could be caused by various process disturbances or equipment malfunctions. The gain scheduling PI approach requires deep process knowledge and analytical skills to develop the adaptation model. This control type performs best when no disturbances or malfunctions are present in the system. The MFA controller tends to perform best during the second phase of the process. At phase II, the recombinant protein is produced, thus the SGR is lower.

The study also revealed that the fuzzy-based control system was stable within the investigated ranges. Considering practical implementation, the developed adaptive control system requires off-gas

analyzers/flow meters and continuous-flow peristaltic pumps, so that the control system's sampling interval stays within defined ranges. This can occur due to the time constants of the measuring devices or gas transport delays. Based on the simulation result, it can be concluded that, in regard to control performance, the described control algorithms are applicable for SGR control in fed-batch biotechnological processes if a substrate feeding rate manipulation and limitation approach are used. Considering the time needed to design and tune the controller, the fuzzy-based PI controller is suitable for practical applications when expert knowledge is attainable in form of a qualitative understanding of the relationships between various important process variables. Such knowledge is usually available from the process operators. The developed controller also performed better when handling disturbances and setpoint-tracking in the first phase of the process. Principally, the proposed approach could be applied in other aerobic cultivation processes with significant oxygen uptake rates. Nevertheless, the fuzzy model needs to be adapted, especially taking into account different OUR ranges and implemented feed-rate controllers. Hence, the fuzzy-based control algorithm is an attractive alternative approach to control the SGR in fed-batch recombinant protein production processes.

In future work, the authors plan to perform further investigations and to split the fuzzy-based adaptation system into two smaller separate fuzzy models for each phase, respectively. This should more accurately approximate the process dynamics and consequently improve the control performance at the second phase.

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