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Keywords: specific growth rate control, batch-to-batch reproducibility, bioreactor control, biotechnological processes

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Review

Practical Solutions for Specific Growth Rate Control Systems in Industrial Bioreactors

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Abstract: This contribution discusses the main challenges related to successful application of automatic control systems used to control specific growth rate in industrial biotechnological processes. It is emphasized that, after the implementation of basic automatic control systems, primary attention shall be paid to the specific growth rate control systems because this process variable critically affects the physiological state of microbial cultures and the formation of the desired product. Therefore, control of the specific growth rate enables improvement of the quality and reproducibility of the biotechnological processes. The main requirements have been formulated that shall be met to successfully implement the specific growth rate control systems in industrial bioreactors. The relatively easy-to-implement schemes of specific growth rate control systems have been reviewed and discussed. The recommendations for selection of particular control systems for specific biotechnological processes have been provided.

Keywords: biotechnological processes; bioreactor control; specific growth rate control; batch-to-batch reproducibility

1. Introduction

Biotechnological processes play an increasingly important role in modern industry and health sectors. Many of the important active pharmaceutical ingredients are recombinant therapeutic proteins produced by the cultivation of genetically modified microorganisms or mammalian cells in bioreactors. These biotechnological processes are highly nonlinear and non-stationary. Therefore, modeling and control of the above bioprocesses are complicated control engineering tasks, especially in industrial recombinant protein production processes, in which high safety requirements and operational restrictions must be secured [1,2]. The goal of this contribution is to review and recommend practical and easily implementable control system schemes for biomass specific growth rate (further referred to as SGR or μ) control in industrial bioreactors. The recommendations are based on an analysis of the existing SGR control solutions and availability of the control schemes suitable for practical implementation in industrial bioreactors. The specific growth rate μ (1/h), is defined as the ratio of the cell's absolute growth rate and the amount of cells:

$$\mu = \frac{dX}{dt}\frac{1}{X} \tag{1}$$

where $X = xV$ (g) is the cell (biomass) amount; x (g/L) is the cell (biomass) concentration; and V (L) is the cultivation broth volume. The SGR is the most important variable in biotechnological processes, which influences the physiological state of microbial culture, production of cell biomass and desired products, and quantity and quality of products [3–8].

The development of relatively simple and reliable methods for SGR monitoring and control in industrial bioreactors is one of the most important control engineering tasks for successful implementation of the Process Analytical Technology (PAT) framework in bioengineering [9,10]. However, to properly exploit the benefits of SGR control systems in microbial and mammalian cell cultivation processes, basic bioprocess variables (temperature, pH, dissolved oxygen concentration, etc.) need to be controlled by commonly available and well-functioning control systems. Unfortunately, in many cases these systems do not ensure sufficient control quality [1,11,12] allowing to further proceed with SGR monitoring and control.

This paper is structured as follows: In Section 2, importance of the control quality of the basic control systems in the biotechnological processes is analyzed. Section 3 introduces important preconditions for implementation of SGR control systems in industrial bioreactors. Section 4 expands on strategies for SGR control suitable for industrial bioreactors. Finally, the authors give recommendations for application of the discussed SGR control solutions in various biotechnological processes.

2. Quality of Basic Control Systems in Industrial Bioreactors

The performance quality of automatic control systems for basic process variables is still low in most industrial microbial and mammalian cell cultivation processes [1,11]. Despite the fact that sophisticated control strategies for microbial cultivation processes are widely discussed in the academic community and research papers [2,13–15], the authors' experience shows that, at present, only simple, conventional automatic control systems are realized in the majority of industrial-scale (bio)reactors [11,16]. This situation is related to a common underestimation of the control systems' importance in improving the productivity and quality of the biotechnological processes. It is also related to the relatively high costs of implementation and maintenance of these advanced control systems and the resultant low acceptance of these systems by plant managers.

Bioreactors are the key operation units in biochemical and biopharmaceutical processes, in which the basic control systems attempt to control the cultivation environment outside of the cell. The commonly controlled variables of the cells' environment are temperature, pressure, pH, and dissolved oxygen concentration. The basic feedback control systems of industrial bioreactors for controlling bacterial cell cultures that produce biopharmaceutical products are presented in Figure 1. The temperature controller manipulates the flow rate of cooling water in the jacket. The pressure inside the bioreactor is controlled by manipulation of the off-gas flow rate. The pH controller manipulates the flow rate of ammonia solution (usually, the acid solution does not need to be added to bacterial cell culture cultivations, unless compensation of base excess is required). The dissolved oxygen controller output is split to manipulate the air flow rate and the agitation speed (at high cell density cultivation the air flow may be enriched by additional oxygen).

Today, the most important industrial cultivations of microbial and mammalian cells are carried out in the fed-batch mode. In fed-batch processes, one or more substrates are fed into the bioreactor during the process. The product remains in the bioreactor until the end of the cultivation cycle. Fed-batch processes overcome substrate inhibition and overflow effects. Such an operational mode allows a high cell density and product concentrations to be achieved [6]. By controlling the substrate feeding rate, the optimal conditions for the biotechnological process can be secured.

To realize the bacterial growth rate control systems, efficient glucose feeding algorithms need to be implemented, and in the mammalian cell cultivation processes, additionally, the feeding rate of glutamine needs to be controlled. It is important to note that modern industrial bioreactors are equipped with inexpensive and reliable devices to measure the composition of aeration gas in the inlet and outlet (fraction of O_2, CO_2) and the molar flow rate, Q. Hence, the oxygen uptake rate (OUR) and the carbon dioxide production rate (CPR) can be calculated from the online measurements as follows:

$$OUR = Q\left(O_2^{in} - O_2^{out}\right), \tag{2}$$

$$CPR = Q\left(CO_2^{out} - CO_2^{in}\right). \quad (3)$$

The above measurements allow an online estimation of the important process variables, OUR and CPR, during bacterial and mammalian cell cultivation processes [17,18]. Because of the lower cell density and respiratory intensity, OUR and CPR measurements based on the off-gas composition may cause larger measurement errors in mammalian cell cultivation processes. As an alternative technique, an OUR estimation using dissolved oxygen (DO) measurements may also be applied [19]. The OUR and CPR are the most important variables for indirect monitoring of the biomass growth rate in industrial bioreactors, as they comprehensively reflect the physiological state and metabolic activity of the aerobic biotechnological processes [1–3,11,20].

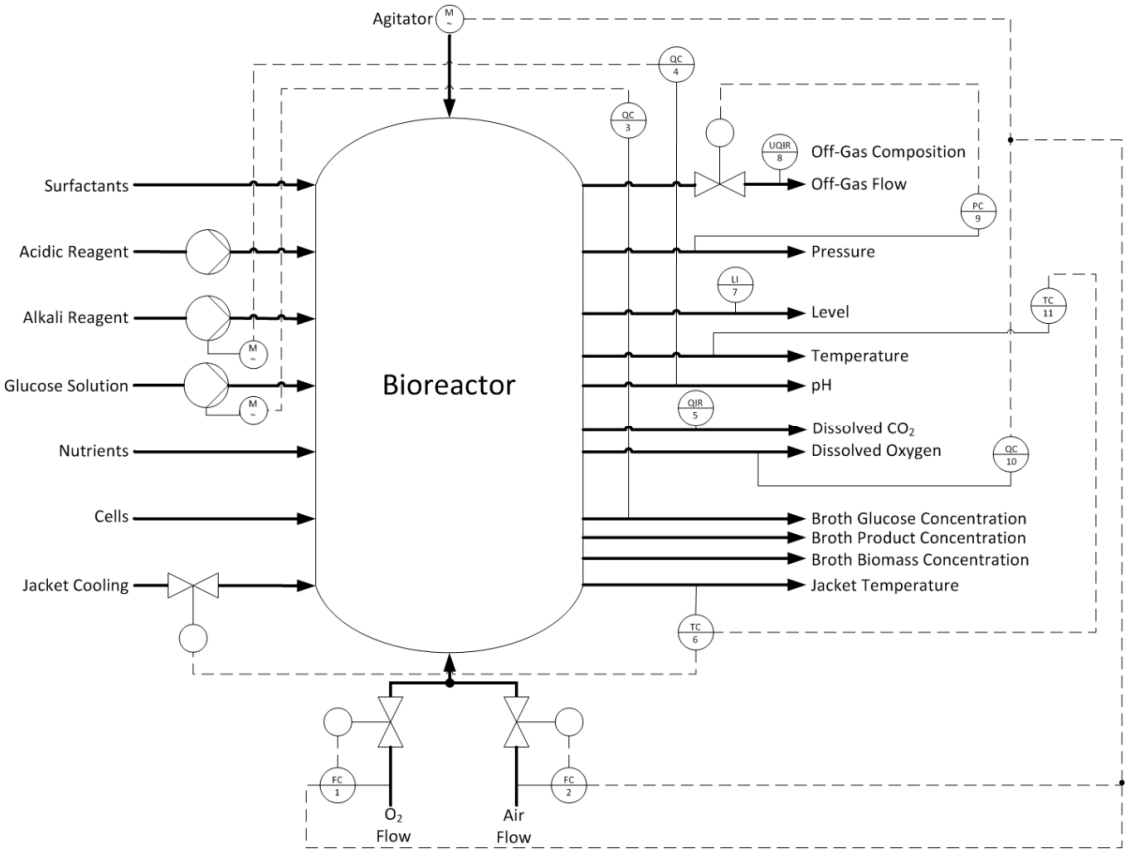


Figure 1. Basic control system loops for a typical microbial cultivation process.

Good performance of the basic control systems improves the batch-to-batch reproducibility/repeatability of the processes [11,21]. The other advantage of a well-controlled bioreactor is the possibility to run the bioreactor at higher capacity or with better efficiency by operating the process closer to physical constraints. Good reproducibility is also an important condition for possible process improvements and modifications, as improvement in reproducibility by means of well-operating control systems allows a reduction in the number of expensive and time-consuming experiments required to compare the performance indices of the modified processes and to optimize the controlled technological regime [1].

The proportional-integral-derivative (PID) controllers are predominant controllers used in the basic control systems of microbial and mammalian cell cultivation processes. Quality of the bioprocess control depends on the complexity of the process dynamics, the process variable measurement noise and errors, tuning of the system controllers, and performance accuracy of the executive devices (valves and speed drives). Dynamics of the particular biotechnological parameter control process can be characterized by three resulting dynamic parameters: dead time, time constant and process gain.

These parameters are commonly used to determine the tuning parameters of a PID controller. Control quality of the bioreactor operation mode critically depends on how well the controllers are set up and tuned to deal with the sources of the process variability [1,2,11,13]. Because of the nonlinearity and nonstationarity of the bioprocesses, proper tuning of the controllers requires appropriate efforts. The performance of PID controllers with fixed tuning parameters are not sufficiently accurate because of the significant variations in the process' dynamics. Consequently, various approaches have been proposed to tune the PID controller parameters in microbial cultivation processes under time-varying operating conditions, including gain-scheduling methods [12,22–24], first-principle models [25], tendency models [26], rule-based fuzzy systems [13], and other techniques [1,2,14,15]. The proposed approaches give a sound theoretical and practical basis to implement adaptive control schemes in bioreactor systems and show that implementation of the adaptive algorithms in basic control systems can significantly increase the performance of the systems. Gain-scheduling methods and tendency models are the most appropriate solutions for improving the quality of basic control systems in microbial cultivation processes because they are relatively simple to implement and pose low requirements on model complexity. The advantages have been extensively discussed and substantiated in previous studies [12,22–24,26]. An important task now is to broaden implementation of these algorithms in industrial bioreactors.

Well-functioning basic control systems create opportunities for further process improvements and also for implementation of the SGR control systems in bioreactors [1,11]. Development of relatively simple solutions to control the specific growth rate in fed-batch processes remains a timely and important task in view of implementing the PAT framework in industrial biotechnological processes.

3. Preconditions for Implementation of SGR Control Systems in Industrial Bioreactors

The basic requirements for SGR control systems designed to control microbial and mammalian cell cultivation processes can be formulated as follows:

- The systems should be as simple as possible and intuitive for the user. Process operators without special modeling/control knowledge should be able to supervise these systems.
- The systems must be based on measurement and control equipment that is currently used standard equipment in industrial bioreactors.
- Development time, cost, and benefits of the systems must be attractive to potential users.

According to the above requirements, most of the solutions for SGR control systems presented in scientific literature [3,6] are not attractive enough for industrial implementation. More complex monitoring and control systems, even if equipped with easy-to-use interfaces, may require retuning, model identification, and maintenance tasks in case the operational modes or microbial cultures have been changed. Often these tasks cannot be carried out by the biotechnology companies alone and may cause additional expenses for outsourcing and production delays. In the authors' opinion, this is the main reason SGR control systems have rarely been used in industrial bioreactors so far.

In this contribution, the authors provide an overview of those SGR control systems that meet the aforementioned requirements. In most widespread control systems, SGR is usually controlled by manipulating the substrate feeding rate [6,27]. In recombinant protein production processes, the temperature of the medium is also used to control cell growth [28]. Despite that the growth rate could be controlled (e.g., by manipulating the dissolved oxygen concentration in cultivation medium [29], temperature of the medium [28] or pH), to date these techniques have not been sufficiently investigated and have not been widely implemented in industrial practice [11].

When the growth rate is controlled by manipulating the substrate feeding rate, the substrate concentration in cultivation medium remains relatively low [30]. This allows avoiding production of the overflow metabolites in some of the most important microbial expression systems, so-called Crabtree-positive organisms, such as *S. cerevisiae* and *E. coli*. The presence of the overflow metabolites,

such as acetate or ethanol, frequently leads to inhibition of both the biomass growth and formation of the proteins.

To ensure controllability and batch-to-batch reproducibility, the SGR needs to be controlled during the process at a level that is lower than the maximum SGR [11]. The maximum available SGR is observed during particular growth phases of the process, when the growth is not limited by substrate concentration [12,31], and depends on the specific culture, medium composition, concentrations of biomass and metabolites, as well as the oxygen transfer capabilities of the bioreactor. It is worth mentioning that direct control of the substrate concentration in bioreactor at the set-point does not guarantee that a constant SGR will be kept. This is because [11]:

- Cell growth at a limited rate occurs under low substrate concentrations. Because of this, online measurements, calibration of the measuring devices, and control of the substrate concentration are difficult to implement in industrial bioreactors.
- Sensor readings of the substrate concentration reflect only the local substrate concentration around the sensor, which may significantly differ from the average concentration in the bioreactor. Therefore, the substrate concentration control system is not able to control the SGR in the entire cultivation medium.

In the majority of recombinant protein production processes, the control objective is to maximize the amount of target protein at the end of the process while maintaining high batch-to-batch reproducibility. To achieve this goal, two steps most often are implemented for SGR control:

- During the first stage of the process, the SGR is kept at a trajectory that is 10–15% below the maximum available SGR.
- During the second stage, the SGR is kept at a trajectory that leads to the maximum specific production rate of the target product. Usually, the level of the SGR kept at this phase is significantly lower compared to that maintained at the first stage.

SGR control systems can be realized using open-loop and closed-loop control systems [6,11]. In the following sections, the authors analyze and evaluate SGR control systems that are best suited for industrial applications. The analyzed and evaluated control solutions are ordered in this review by their complexity (i.e., starting with the simplest open-loop systems and ending up with the control systems that employ cascade control schemes and SGR estimators).

4. Schemes for SGR Practical Control Systems

4.1. Open-Loop SGR Control Systems

The majority of industrial fed-batch microbial cultivation processes are operated using open-loop SGR control systems [11], in which the time profile of the substrate feeding rate is calculated using simple mass-balance models and a desired time profile of the SGR during the process. The desired SGR values, μ_{set}, can be described by the following equation:

$$\mu_{set} = \begin{cases} \mu_{set_1} = (0.85 \ldots 0.90)\mu_{max} \; for \; growth \; phase, \\ \mu_{set_2} = \mu_{opt} \; for \; production \; phase. \end{cases} \quad (4)$$

Based on the desired set values for SGR, the corresponding substrate feeding rate can be determined. Accumulation of the total biomass during cultivation and the substrate feeding rate for both stages of the process can be estimated from simple mass-balance equations:

$$\frac{dX}{dt} = \mu_{set_i} X, \;\; i = 1, \; 2. \quad (5)$$

The amount of biomass accumulated in the growth stage can be calculated from the equation

$$X(t) = X_0 e^{\mu_{set_1} \, t}, \quad (6)$$

and the amount of biomass accumulated in the production stage can be calculated from the equation

$$X(t) = X_0 e^{\mu_{set_1} \ t_g} \cdot e^{\mu_{set_2} \ (t-t_g)}, \tag{7}$$

where t_g (h) is the end time of the growth stage.

Using the predicted time trajectories of the biomass accumulation, *X(t)*, the substrate feeding rate $F_1(t)$ in the growth stage can be derived from the dynamic mass-balance equation for the substrate under steady-state conditions [2,6,8,20,30], which results in the following equation

$$F_1(t) = \frac{X_0 e^{\mu_{set_1} \ t} \mu_{set_1}}{Y_{xs1} S_F}, \tag{8}$$

and the substrate feeding rate $F_2(t)$ in the production stage can be estimated from the equation

$$F_2(t) = \frac{X_0 e^{\mu_{set_1} \ t_g} e^{\mu_{set_2} \ (t-t_g)} \mu_{set_2}}{Y_{xs2} S_F}, \tag{9}$$

where X_0 (g) is the total amount of biomass in the bioreactor at the beginning of cultivation process; *X(t)* (g) is the time trajectory of the total biomass accumulated during the process; S_F (g/L) is the concentration of the substrate in the feeding solution; and Y_{xs1} and Y_{xs2} (g/g) are the yields of biomass on substrate in the growth and production phases, respectively. In substrate-limited processes, the substrate concentration in the bioreactor is low. Therefore, this concentration is not taken into account in Equations (8) and (9).

When the SGR control algorithm based on Equations (8) and (9) is developed, implementation of the control system is straightforward. For this purpose, only an actuator to dose the feeding substrate to the bioreactor is needed. For some recombinant protein production processes, the yield of biomass on substrate can be different in the growth (Y_{xs1}) and the production stages (Y_{xs2}). In such cases, the yields must be identified from experimental data for the particular process phase and must be taken into account when using Equations (8) and (9) to estimate the substrate feeding rates. Additionally, μ_{max} and μ_{opt} may vary during the process because of the increasing concentrations of metabolites, biomass, and other process variables. In this case, $\mu_{max}(t)$ and $\mu_{opt}(t)$ should be presented as time profiles, and a numerical integration procedure to predict the biomass growth and substrate feeding time profiles needs to be applied.

The substrate feeding time profiles estimated from Equations (8) and (9) can be directly used for implementing the open-loop SGR control systems in various biotechnological processes [6,7,22,30,32,33]. Certainly, more sophisticated bioprocess models and optimization procedures can be used to determine the feeding rate control algorithms in open-loop SGR control systems. These methods are widely reviewed and analyzed in many research and academic papers [3,6,8,27,34,35]. However, implementation of more sophisticated procedures in industrial bioprocesses requires specific knowledge in process modeling and efforts to develop more accurate models. Consequently, application of complex methods in industrial environment is not a commonplace.

The SGR open-loop control systems based on Equations (8) and (9) are easy to implement and do not require additional measurements. On the other hand, open-loop systems do not compensate for process disturbances. Consequently, possible variations in the substrate concentration of the feeding solution or deviations of the feeding flow rate are not compensated. These disturbances can decrease the performance of the biotechnological process. In the next sections, the authors analyze and provide relatively simple and already existing solutions to overcome these problems.

4.2. SGR Control Systems Based on CPR/OUR Estimations

Reliability and accuracy of SGR control can be increased by employing closed-loop control systems. One of the simplest closed-loop SGR control systems is proposed in Reference [27]. Here, based on a

simplified assumption that the carbon dioxide production rate (CPR) during the process is in a linear relationship with the biomass growth rate

$$CPR(t) = \alpha\mu(t)X(t), \tag{10}$$

the specific growth rate μ can be estimated using the following equation

$$\mu(t) = \frac{CPR(t)}{\int_0^t CPR(\tau)d\tau}, \tag{11}$$

where α is a model parameter, and τ is the integration time variable.

If real-time estimation of SGR is available, feedback control systems can be developed to automatically track a desired SGR time profile by manipulating the substrate feeding rate.

Results of the SGR control obtained in Reference [36] show that an acceptable control quality can be obtained by applying a typical control system based on PI controllers. To achieve better control quality, it is straightforward to adapt the controller parameters to the time-varying dynamics of the controlled process by applying gain-scheduling algorithms mentioned in Section 2 and using the *CPR* signal as a scheduling variable. The main drawback of the analyzed control approach is that, during SGR estimation, an assumption is made that the *CPR* during the process is proportional to the absolute biomass growth rate. In fact, it is known that more accurate results may be achieved if the Luedeking–Piret-type relationship is applied to correlate the *CPR* and the biomass growth rate [20,37]. This relationship additionally takes into account the *CPR* fraction that is related to the maintenance of the cell's vital functions and accounts for a significant part of the total *CPR* (for instance, in high-cell-density bacterial cultivation processes). Figure 2 shows the simulated trajectories of the biomass growth and the *CPR* of the recombinant *E. coli* cultivation process in a 1 m^3 volume bioreactor as well as the comparison of the actual and the estimated SGRs. The latter is calculated from Equations (10) and (11). The actual *CPR* of the process is modeled using the equation

$$CPR(t) = \alpha\mu(t)X(t) + \beta X(t), \tag{12}$$

where parameter β determines the *CPR* fraction related to maintenance of the cell's vital functions.

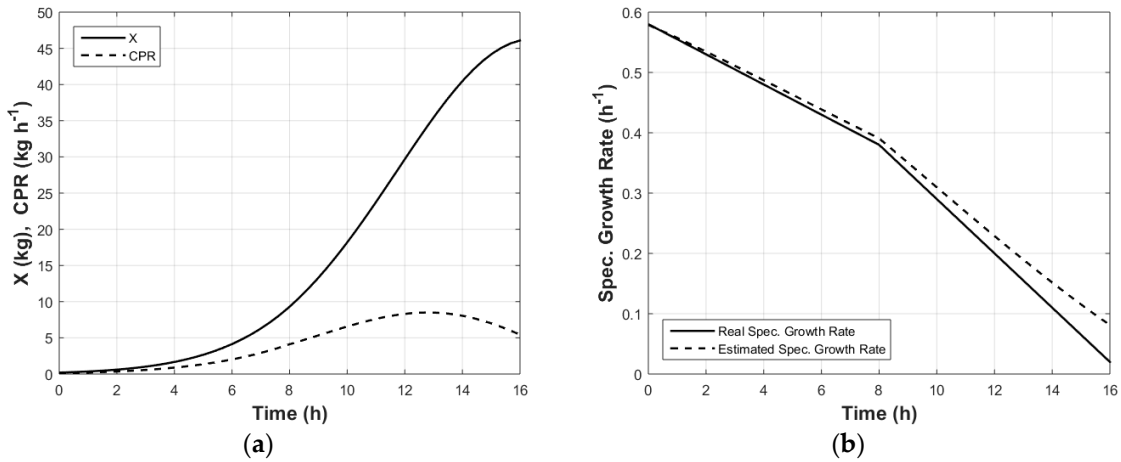


Figure 2. Simulated trajectories of the biomass growth and carbon dioxide production rate (*CPR)* (**a**), and the trajectories of the real specific growth rate (SGR) and that estimated from Equation (7) (**b**) in a typical recombinant *E. coli* cultivation process in a 1 m^3 bioreactor.

In the simulation experiment, values of the parameters α and β were used that are typical for recombinant *E. coli* cultivation processes (α = *0.9 (gCO₂/gX)*, and β = *0.1 (gCO₂/(gX·h))*), induction at

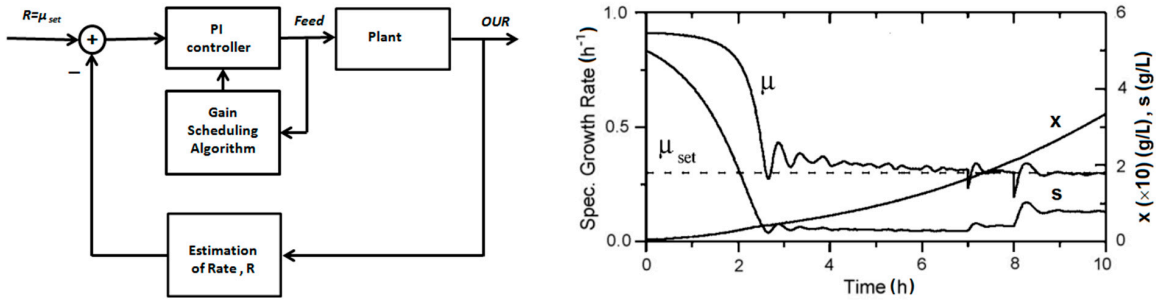
$t = 8\ h$) [38]. The simulation results, presented in Figure 2, show that the estimated SGR deviation from the real trajectory increases with an increasing amount of biomass (the estimated rate at the end of the process is *0.05 (1/h)* higher than the real one). Hence, it is advantageous to introduce empiric correlations correct the estimated SGR when applying this method in high-density cultivation processes. The magnitude of correction should be defined from earlier cultivation experiments.

To estimate SGR, *OUR* data can also be used. However, the measurements related to *OUR* estimation may be corrupted by the noise related to the off-gas composition, pressure, and the gas flow rate fluctuations if additional oxygen is used enrich the aeration air. Therefore, to control the high-density cultivation processes, it is recommended to use *CPR* data in SGR estimation relationships.

A more accurate SGR control system based on *OUR* or *CPR* measurements is proposed in References [25,39]. Realization of the proposed control system does not require a mathematical model and *a priori* knowledge of the culture of the microorganisms under control. It can be realized using standard programmable controllers/measurement devices and is well suited for control of industrial biotechnological processes. In the cited contributions, it is shown that if the substrate feeding rate is manipulated to control *OUR* during the process, in such a way that the *OUR* data-based ratio R

$$R = \frac{dOUR}{dt}\frac{1}{OUR}, \tag{13}$$

is stabilized at the desired SGR set-point $R = \mu_{set}$, then the specific growth rate μ will asymptotically approach the set-point μ_{set} and will be controlled at that point. For control of the ratio R, the PI control algorithm was recommended, and controller gain was adapted to the time-varying dynamics of the controlled process using the gain-scheduling approach with the feeding rate as the scheduling variable. The block-scheme of the SGR control system and the simulation results of the system's performance are presented in Figure 3. The simulation and experimental investigation tests of the proposed SGR automatic control system have shown a stable performance and sufficiently accurate control of the SGR under stepwise changes to the process parameter values and high-level noise of the feedback signal measurements [25,39]. This control system can be efficiently applied in controlling biotechnological processes, in which the SGR set-point is constant or changes slowly. For realization of the system, either *OUR* or *CPR* online estimates can be used.



(**a**) (**b**)

Figure 3. Block-scheme of the SGR control system (**a**) and the simulation results of the system performance (**b**). Reproduced with permission from D. Levišauskas, Biotechnology Letters; published by Springer Nature, 2001.

It should be stressed that the SGR control systems based on Equation (11), when applied in high-density cultivation processes, cause noticeable deviations at high cell concentrations. SGR control systems based on Equation (13) are less efficient when tracking time-varying SGR set-point profiles.

In the next sections, more complex control systems are discussed that overcome the above shortcomings.

4.3. SGR Control Systems Based on CPR/OUR Estimations and the Mass of CO2/O2 Produced/Consumed During Cultivation

Robust control of the SGR is a crucial problem when designing an efficient process, in which the SGR is to be controlled at the value $\mu_{set} < \mu_{max}$ in order to secure reproducibility of the processes. However, the already discussed SGR closed-loop control systems have two shortcomings: (a) for system implementation, an online estimation of the μ-values is required, and (b) high batch-to-batch reproducibility is not guaranteed. For example, if disturbances occur during a process (e.g., variation in the initial amount of biomass X_0) or in the instrumentation (e.g., if the substrate feeding is shortly interrupted), they cause slight deviations in the biomass growth trajectory from the desired trajectory, and such an offset cannot be eliminated later on, even if the controller exactly tracks the predefined μ-profile. An approach to cope with the disturbances that cause process reproducibility problems is proposed in Reference [40]. In this work, a desired SGR time profile $\mu_{set}(t)$, the initial amount of biomass X_0, and Equation (1) were used to estimate the biomass growth time profile $X(t)$ during the process. If the biomass growth profile $X(t)$ can be tightly controlled by manipulating the substrate feeding rate, the corresponding SGR profile will follow the desired $\mu_{set}(t)$ profile. This control system is more robust as compared to direct SGR control systems, as the short-term disturbances that occur in the control equipment and the process itself are compensated by controlling an integral variable—the amount of accumulated biomass $X(t)$. However, implementation of the above control system requires development of a reliable soft-sensor for the online estimation of the amount of accumulated biomass during the process. Therefore, the $X(t)$ online estimation problem complicates the practical realization of this control approach. To eliminate this shortcoming, simplified SGR control systems were developed and experimentally tested in bacterial and mammalian cell cultivation processes [41–43]. The main idea behind these control systems is to use the predetermined time profiles of $CPR_{set}(t)$ as the system's time-varying set-point, and the mass of $CO_2(t)$ produced during the process (mCO_{2set}) as an indirect metric for SGR control purposes. $CPR(t)$ is stoichiometrically related to the SGR and the biomass (Equation (9)), and the integral of this equation gives the mass $mCO_{2set}(t)$ produced during the process.

By manipulating the substrate feeding rate to control the predetermined set-point time profile $mCO_{2set}(t)$, the control system indirectly maintains the desired SGR during the process. The structure of the discussed cascade control system is depicted in Figure 4. The PI controller of the inner loop controls the $CPR_{set}(t)$ time profile, and the PI controller of the outer loop controls the set $mCO_{2set}(t)$ profile.

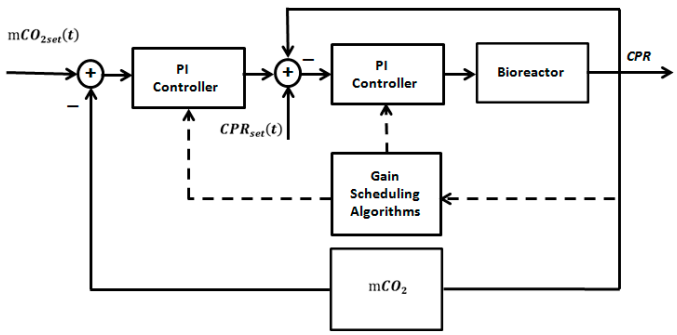


Figure 4. Cascade control system for indirect SGR control based on predetermined $CPR_{set}(t)$ and $mCO_{2set}(t)$ time profiles.

If the controlled process is tightly kept on the $CPR_{set}(t)$ and $mCO_{2set}(t)$ time profiles, the process will also follow the desired SGR time profile. The proposed control system ensures good quality of the SGR control, and, because of the cumulative nature of the set-point variable $mCO_2(t)$, random disturbances do not significantly distort the course and reproducibility of the process.

Implementation of the proposed control system can be realized in the following steps:

- Choose a rational $\mu_{set}(t)$ time profile for the process. A proper profile can be estimated from expert knowledge, mathematical model-based process optimization results, or from the analysis of a successful "golden batch" experiment.
- Choose an appropriate inoculum size (initial amount of the total biomass X_0) for the process and estimate the biomass growth time profile $X(t)$ using the $\mu_{set}(t)$ profile, Equation (4), and a numerical integration procedure.
- Estimate the $CPR_{set}(t)$ time profile using Equation (10) and the identified parameter values α and β. Note that the above parameter values may be different for the biomass growth and product formation stages.
- Integrate the $CPR_{set}(t)$ time profile to get the corresponding profile $mCO_{2set}(t)$ for the controlled process.
- Control the process by tracking the estimated profiles $CPR_{set}(t)$ and $mCO_{2set}(t)$. Control is realized using the cascade control system that manipulates the substrate feeding rate.

Various realizations of the above control system have been investigated by computer simulations of the system's performance and by controlling real processes of recombinant *E. coli* and mammalian cells (CHO) [18,41,42]. Typical results of the applied control system for controlling the recombinant *E. coli* fed-batch cultivation processes over six runs are presented in Figure 5. The laboratory-scale experimental results show that the proposed control approach leads to a stable and robust behavior of the controlled process. It should also be stressed that small variations in the initial amount of biomass X_0 and short instrumentation disturbances do not significantly affect the reproducibility of the process.

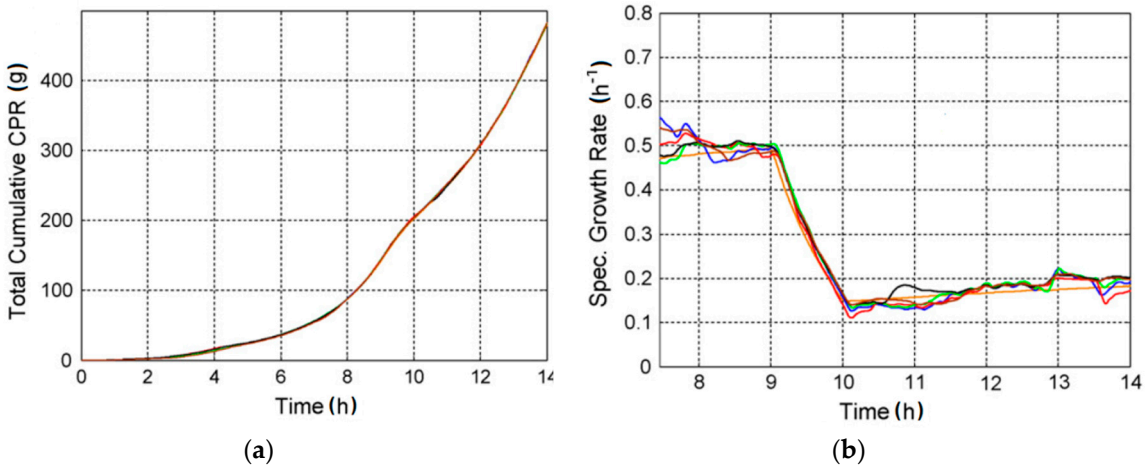


Figure 5. Typical experimental results of the total cumulative CPR (**a**) and SGR indirect control ($\mu_{set}(t)$ = 0.5 1/h at the first process stage and $\mu_{set}(t)$ = 0.175 1/h at the second stage) (**b**) during the recombinant *E. coli* cultivation process. Reproduced with permission from M. Jenzsch et al. J. of Biotechnology; published by Elsevier, 2007.

Because of the significant changes in the process dynamics during cultivation, it is possible to improve control quality of the cascade control system by adapting controller parameters. Tuning parameters of the PI controllers can be adapted to time-varying dynamics of the controlled process using the gain-scheduling approach. The controller adaptation scheme using the gain-scheduling algorithm is shown in Figure 4 by the dashed lines. In the gain-scheduling algorithms, one can use *CPR* or *OUR* measurements as the gain-scheduling variables.

Instead of using the *OUR(t)* or *CPR(t)* time profiles, the performance of the inner control loop of the cascade control system can also be improved by implementing the SGR estimator, developed from Equations (12) and (13) [44]. Investigation results presented in Reference [44] have shown that the control system with the SGR estimator outperforms the control system depicted in Figure 4 when the controlled process is affected by disturbances to the substrate feeding rate. On the other hand,

implementation of the modified control system requires additional calculations related to online estimation of the SGR.

The structure of the SGR control system presented in Figure 4 may be used as a basis for development of closed-loop control systems for controlling the processes of various microbial cultures in industrial bioreactors. Because it is technically simple to implement and possible to improve batch-to-batch reproducibility, this system could be used as a benchmark to compare the control quality of various SGR control systems and to evaluate their potential implementation in industrial bioreactors.

5. Concluding Remarks and Recommendations

In recent years, numerous research papers have been published, in which original solutions and sophisticated control techniques were developed for the automatic control of microbial and mammalian cell cultivations processes. However, the majority of the proposed control systems are too complicated to be attractive for robust control of industrial biotechnological processes. Therefore, the well-known statement of Luyben [16], "Complex elegant control systems look great on paper but soon end up on 'manual' in an industrial environment", is also valid for the majority of the control systems developed for biotechnological processes.

In this paper, relatively simple control approaches that can be applied in microbial and mammalian cell cultivation processes are discussed and recommended for practical application. The reviewed algorithms and systems designed for indirect control of the specific growth rate can significantly increase robustness and batch-to-batch reproducibility of industrial-scale biotechnological processes. The recommended control algorithms and systems are based on *CPR* or *OUR* online measurements and on the total mass of oxygen consumed or the total mass of carbon dioxide produced during the process. In the case when additional oxygen is used during the processes, it is recommended to use the *CPR* and mCO_2 signals in the control system algorithms because of their lower estimation errors compared to those when using *OUR* and mO_2 signals. To estimate oxygen uptake and carbon dioxide production rates, several industrially well-established gas analyzers and mass flow meters are available. Basic instrumentation for installation of the SGR control systems, the online gas analyzer, combines parallel measurement of CO_2 and O_2 concentrations in the off-gas using two space-saving sensors. The analyzer can be used both for lab- and industrial-scale bioreactors. In the industrial gas analyzers, compensators for gas pressure and humidity are incorporated. Consequently, these analyzers ensure good precision and reliability of the measurements.

The available instrumentation and discussed control methods and systems provide a possibility for wider application of SGR control in biotechnological processes. At the very beginning of the process, accuracy of the indirect measurements is usually low and insufficient to track exactly the SGR set-point time profile in closed-loop control systems. Consequently, it is recommended to start the process using the feeding rate open-loop control strategies determined by Equations (8) and (9) and, after three to four hours, to switch the SGR control to the closed-loop control system. For the low-density cell cultivation processes, the SGR control system based on Equation (11) is recommended. For processes, in which the SGR set-point is kept constant, the control system based on Equation (13) (Figure 3) is well suited. For more advanced applications, the SGR control system presented in Figure 4 is recommended. The above system can be applied as a benchmark to compare the control quality of various SGR control systems.

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