Chapter

Practical Approaches to the Control of Milk Fermentation with Kefir Grains

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Abstract

In the chapter, milk fermentation for kefir production is studied. The traditional kefir production process based on inoculating kefir grains into milk is considered. The quality and quantity of the produced kefir also depend on the dynamics of the fermentation process. The chapter presents the design and synthesis of the closed-loop control system in which changing the bioreactor's temperature is used to control the time course of the concentration of dissolved CO_2. In the chapter: (1) a nonlinear dynamic mathematical model of the fermentation process, which allows evaluating the influence of the bioreactor's temperature on the dynamics of the fermentation process, is presented; (2) the design and synthesis of a conventional linear control system with constant parameters are carried out; (3) an adaptive control system that enables the tracking of the courses of the quantities of the fermentation process to the desired reference trajectories without the time-consuming preliminary identification of the parameters of the fermentation process model is developed. The numerical, experimental, and analytic outcomes of the study are presented.

Keywords: milk fermentation, batch bioreactor, mathematical modeling, control system design and synthesis, linear control, model reference adaptive control

1. Introduction

In the chapter, milk fermentation for kefir production is studied. The traditional kefir production process based on inoculating kefir grains into milk is considered. Characteristics, quality, and quantity of the produced kefir depend on the used microorganisms and substrate, but they also depend on the time course of the fermentation product concentration [1]. Although different types of bioreactors can be used for kefir production, batch bioreactors are highly practiced [2]. The specific operation of batch bioreactors allows their simple construction, which is returned with easy maintenance and low production costs of bioreactors [3]. Therefore, batch bioreactors are the most widely used bioreactors. Unfortunately, in batch bioreactors, it is not possible to add or remove individual substances during operation and thus influence the course of the fermentation process [4]. This represents a major

limitation in the use of existing batch bioreactors. Fermentation processes in the existing industrial and laboratory batch bioreactors run independently (autonomously). The courses depend only on the initial concentrations of substances, and once the fermentation processes have been started, it is no longer possible to influence the execution of the fermentation processes. This problem is well-known in the industrial and academic environment. There are many publications showing this problem [2–4] and possible solutions [5–7].

This chapter presents another option to control the time course of the fermentation process. The possibility of controlling the time course of the fermentation process by changing the bioreactor's temperature instead of adding individual substances during the operation is investigated in the chapter thoroughly.

In the study's initial phase, we also reviewed relevant journal publications considering fermentation process control published in the period 2000–2020. The purpose of the review was to determine the direction of research in this area and to analyze the existing solutions to this problem. We used established research databases in both related fields, that is, Electrical Engineering/Control and Bioprocess Engineering/Bioreactors. As expected, we found a lot of recent interesting work in the field of Continuous Bioreactor Control and in the field of fed-batch bioreactor control. Some interesting articles concerning the use of advanced control techniques in continuous bioreactors are presented in Refs. [5–11]. The implementation of advanced control methods for fed-batch bioreactors can be found in many articles; some of them are [12–15]. Unexpected and very interesting, however, was the disclosure that there are very few publications dealing with the control of batch bioreactors. We found only a few papers where the control of the batch bioreactors was realized. In both cases, the manipulation of airflow of oxygen concentration was used for control. In Ref. [16], gain scheduling control was used, and a conventional PI control strategy was proposed in Ref. [17]. This was our additional motivation to work more in-depth and intensively in the field of control of fermentation processes in batch bioreactors.

The problem of control of fermentation processes in batch bioreactors is frequent and up-to-date. Based on the increasing interest in a suitable solution and on the basis of already existing knowledge presented in publications, we started with the multidisciplinary multiannual cooperation of three faculties. Our work was divided into three stages: (1) design of the control system, which will enable assure the prescribed operation in batch bioreactors; (2) derivation of the mathematical model of the fermentation process in batch bioreactors which also describes the impact of operating conditions on the execution of the fermentation process and will be suitable for the analysis and simulation of the fermentation process and for the development of the control system; (3) synthesis of the advanced control system, which will approve a noncomplicated and practical implementation. The work done has been addressed in some publications. The use of the heating system for control of the fermentation process in batch bioreactors was presented in Refs. [18–22], and use of the stirrer system in Refs. [18, 23]. In this chapter, the continuation of work in the field of kefir production control is presented and the findings are summarized and collected.

The basic hypothesis of our work was that the fermentation process in batch bioreactors can be controlled by changing the temperature in the bioreactor. In this way, it is possible to achieve the desired time courses of concentrations of substances in the fermentation process and thus influence the quality and quantity of the fermentation product. To achieve this goal, we need a control system, which must be simple enough to be implemented in a batch bioreactor. It is also necessary that the tuning of the controller parameters is automatic and that the users of the bioreactors will not need

additional knowledge and time-consuming setting of the parameters of the control system. The second hypothesis was that a control system that will meet the set requirements can be realized based on the theory of adaptive systems.

For this purpose, the chapter presents a nonlinear dynamic mathematical model of the fermentation process, which allows evaluation of the influence of operating parameters (stirrer speed, temperature) on the dynamics of the fermentation process. Based on this model, the design and synthesis of a conventional linear control system with constant parameters can be carried out. Accurate knowledge of the fermentation process model parameters is required for tuning the parameters of a conventional linear controller, which significantly reduces the usability of such a control system. The chapter's emphasis is on the development of an adaptive control system that enables the following of the responses of the quantities of the fermentation process to the desired reference trajectories without the time-consuming preliminary identification of the parameters of the fermentation process model. The main contribution of the chapter is the presentation of the development of a control system that will enable the control of the fermentation process for kefir production even in uncomplicated batch bioreactors. The developed control system confirmed both hypotheses.

The chapter is organized into nine Sections. In Section 2, a considered milk fermentation process with kefir grains is described. In Section 3, the available laboratory system consisting of a batch bioreactor with a controlled heating/cooling system, measurement equipment, and equipment for data acquisition and control is presented. In Section 4, a mathematical model of the fermentation process in the batch bioreactor is described. Results obtained with the derived mathematical model are shown in Section 5. A batch bioreactor's closed-loop control system is developed based on the knowledge gained from the mathematical model analysis. The design and synthesis of the control system are carried out in two ways: By using the conventional linear control theory and on the basis of the advanced adaptive control theory, both concepts are shown in Section 6. The results of both control approaches are presented in Section 7. Discussion, comments, and concluding remarks are offered in Sections 8 and 9.

2. Controlled fermentation process

The given study deals with milk fermentation. The release of CO_2 is studied, and the focus is on fermentation with kefir grains. Inoculum-initiated milk fermentation lasts approximately one day. During this time, homofermentative lactic acid streptococci grow fast, at the beginning producing a decrease in pH. Reduced pH causes a decrease in the number of streptococcus and at the same time accelerates the growth of lactobacilli. Yeasts and increased temperature have a positive effect on the growth of heterofermentative streptococci. These have a significant influence on the aroma of the product.

Before the considered fermentation process, kefir grains were activated for five consecutive days (daily washing with cold water and after that putting in milk at room temperature). For starting of the fermentation process the pasteurized fat milk was preheated and inoculated with activated grains. By using different kefir grains, the fermentation processes with different dynamic and static characteristics were performed. The considered fermentation processes are nonlinear dynamic processes that require sophisticated control algorithms to achieve desired behavior and consequently quality fermentation products.

3. Used lab apparatus

A laboratory batch bioreactor with additional equipment was applied to show the applicability of the proposed practical approach for the control of the kefir fermentation process. This laboratory system was also used for the thorough analysis and modeling of the fermentation process. Before the implementation of the control system, a systematic analysis through the set of experiments was carried out. First, a set of experiments with constant operating conditions was performed. Then, a multitude of tests with the changeable temperature in the bioreactor was done. The goal of these experiments was to discover the impact of the temperature of the fermentation substances on the dynamics of the fermentation process. During these experiments, the input quantity of the analyzed fermentation process was the temperature in the bioreactor, and the output quantity was the dissolved CO_2, which represents the controlled fermentation product.

3.1 Batch bioreactor

Fermentation processes were performed in the reaction calorimeter Mettler Toledo RC1e, which was applied as a batch bioreactor. A comprehensive explanation of RC1e is presented in Ref. [22].

3.2 Integrated system for heating and cooling of fermentation mixture

The tested bioreactor was originally provided with the system for heating and cooling (heating/cooling system). The silicone oil was implemented as a transfer liquid, which is injected into a closed circulation system through the double jacket of the reactor. The heating/cooling system is completed with the closed-loop temperature control system. A proportional-integral controller was used. The heating/cooling system enables the adjustment of the bioreactor's temperature in the area from 5 to 50° C. Due to the controller's integral characteristic, the control system operates without steady-state error for a constant reference temperature. The lag in the control system is substantial in comparison to the lag of the fermentation process. The heating/cooling system was modeled with the 1st order lag term with unit gain and time constant $T_{\vartheta cs}$ = 0.1 h.

3.3 Dissolved CO_2 measurement

The choice of the controlled plant output quantity is decisive for the selection of the control approach and for the practical realization. The selected quantity must comprehend the essential information about the fermentation process. It is also required the unproblematic, accurate, and fast realization of its measurement.

For the control and monitoring of the fermentation processes, the measurements of dissolved oxygen and cell culture are fundamental. Oxygen concentration is very important because it correlates with growth and metabolite. Another quantity important in the execution of the fermentation process is the concentration of the biomass.

During laboratory experiments or operating in an industrial environment, it is very difficult to measure dissolved oxygen and/or cell culture concentrations. Therefore, in

the presented contribution, we have shown how we can use the measurement of dissolved CO_2 concentration instead of measuring these two quantities [24]. CO_2 is the outcome of microorganism metabolism and represents a good indicator of the course of fermentation. A good feature of using CO_2 measurement as an indicator of the fermentation process is the ease of CO_2 measurement. CO_2 is homogeneously distributed in the reactor mixture, the sensors are accurate, reliable, have a long lifespan, their measurement curve is known [25]. The CO_2 measurement is fast and suitable for real-time control systems.

3.4 Equipment for data acquisition and control

The ISE51B ion-selective electrode was used for the measurement of the CO_2 concentration [23]. Electrode potential response to CO_2 concentration is in a semilogarithmic scale, a straight line over two decades of the concentration (5·10–4 g/L to 2·10–2 g/L). For the acquisition of the measured signal of the dissolved CO_2 concentration, the hardware SevenMulti from Mettler Toledo together with LabX direct software was implemented. The control system was realized by means of dSpace 1103 device [22]. Block diagrams of the laboratory measurement and control system are shown in **Figures 1** and **2**.

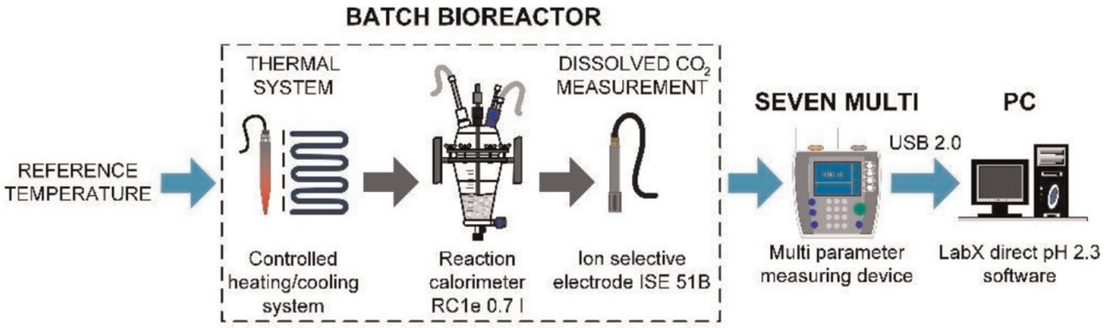

Figure 1.
Laboratory batch bioreactor system for milk fermentation process analysis and modeling [22].

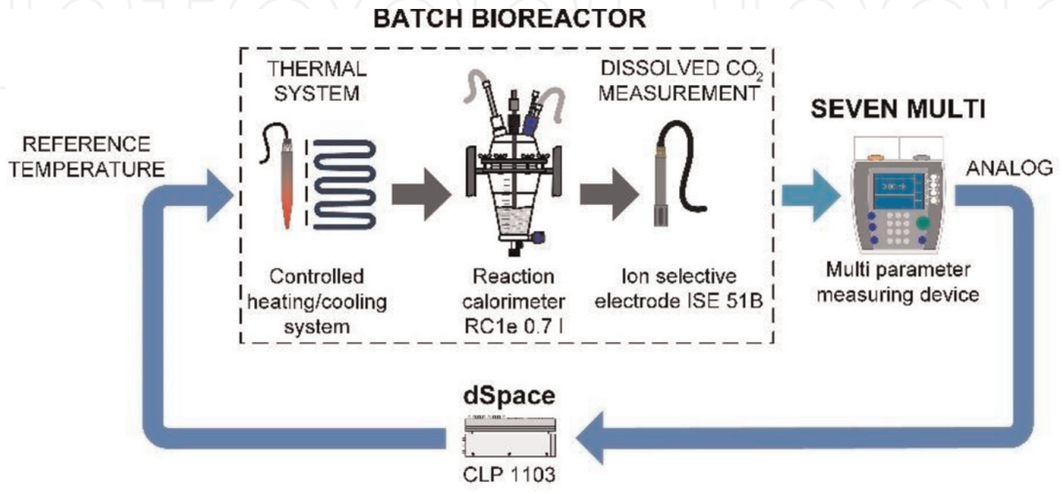

Figure 2.
Laboratory batch bioreactor system for the control of the milk fermentation process [22].

4. Mathematical model of the fermentation process dynamics in a batch bioreactor

The fermentation process is a biological process where the microorganisms cause the source substrate to break down into the final product, that is, the microorganisms consume substrate and, during the growth, produce the product. Microorganisms, substrates, and products are basic substances in all fermentation processes. In the presented study, the fermentation processes in batch bioreactors will be considered.

The fermentation process is a complex process. It can be described by a nonlinear and time-dependent mathematical model with a partially known structure and unknown and time-varying parameters. In the references, we can find different mathematical models of different grades of complexity, which can be used for the description of the fermentation processes in batch bioreactors. Most models are based on the mass balances of the microorganisms, substrate, and fermentation product. A fundamental and most frequently applied mathematical model describes the dynamics of the fermentation process in a batch bioreactor with a system of three nonlinear differential equations. The differential equations are used to calculate the concentration of the substances in the batch bioreactor: microorganisms, substrate, and fermentation product. The differential equations are represented by the Eqs. (1)–(3):

$$\dot{x}_1(t) = \frac{\mu_m \left[1 - \frac{1}{P_i} x_3(t)\right] x_2(t)}{S_m + x_2(t) + \frac{1}{S_i}[x_2(t)]^2} x_1(t) \tag{1}$$

$$\dot{x}_2(t) = -\frac{\mu_m \left[1 - \frac{1}{P_i} x_3(t)\right] x_2(t)}{S_m + x_2(t) + \frac{1}{S_i}[x_2(t)]^2} x_2(t) \tag{2}$$

$$\dot{x}_3(t) = \left[\alpha \frac{\mu_m \left[1 - \frac{1}{P_i} x_3(t)\right] x_2(t)}{S_m + x_2(t) + \frac{1}{S_i}[x_2(t)]^2} + \beta\right] x_3(t) \tag{3}$$

where the following symbols for the concentration of the fermentation process substances are used:

$x_1(t)$: microorganisms' concentration (g/L),

$x_2(t)$: substrate's concentration (g/L),

$x_3(t)$: fermentation product's concentration (g/L),

and the following symbols for the parameters of the mathematical model are introduced:

μ_m: maximum growth rate of the microorganisms (h^{-1}),

P_i: inhibition constant of the fermentation product (g/L),

$S_{m:}$ saturation constant of the fermentation substrate (g/L),

S_i: inhibition constant of the fermentation substrate (g/L),

A: correlation parameter among yield of fermentation product and growth of microorganisms and.

β: correlation parameter that denotes the yield of fermentation product independent of the growth of microorganisms (h^{-1}).

The presented mathematical model can be used for the simulations of the fermentation processes in the batch bioreactors in a case when the temperature stays constant

during the execution of the fermentation processes. In the case of the changeable temperature, the presented mathematical model does not allow the simulations of the fermentation processes. In this case, the enhanced mathematical model must be used. The enhanced mathematical model, which considers the influence of the bioreactor's temperature changes on the dynamics of the fermentation process, is described by a system of four differential Eqs. (4)–(7) [20]:

$$\dot{x}_1(t) = \frac{\mu_{\mathrm{m}\vartheta}(t)\left[1 - \frac{1}{P_{\mathrm{i}\vartheta}(t)}x_3(t)\right]x_2(t)}{S_{\mathrm{m}} + x_2(t) + \frac{1}{S_{\mathrm{i}}}[x_2(t)]^2}x_1(t) \tag{4}$$

$$\dot{x}_2(t) = -\frac{\mu_{\mathrm{m}\vartheta}(t)\left[1 - \frac{1}{P_{\mathrm{i}\vartheta}(t)}x_3(t)\right]x_2(t)}{S_{\mathrm{m}} + x_2(t) + \frac{1}{S_{\mathrm{i}}}[x_2(t)]^2}x_2(t) \tag{5}$$

$$\dot{x}_3(t) = \left[\alpha\,\frac{\mu_{\mathrm{m}\vartheta}(t)\left[1 - \frac{1}{P_{\mathrm{i}\vartheta}(t)}x_3(t)\right]x_2(t)}{S_{\mathrm{m}} + x_2(t) + \frac{1}{S_{\mathrm{i}}}[x_2(t)]^2} + \beta\right]x_3(t) \tag{6}$$

$$\dot{x}_4(t) = \frac{1}{T_{\vartheta\mathrm{cs}}}[x_4(t) + u(t)] \tag{7}$$

where added to the symbols in Eqs. (1)–(3):

$x_4(t)$ stands for the temperature of the contents of the bioreactor (°K),

$u(t)$ denotes the reference temperature of the bioreactor's temperature control system (°K),

$T_{\vartheta\mathrm{cs}}$ marks the time constant of the 1st order lag term, which represents the mathematical model of the controlled heating system (h) and.

$\mu_{\mathrm{m}\vartheta}(t)$, $P_{\mathrm{i}\vartheta}(t)$ denote functions that represent the impact of the temperature of the contents of the bioreactor on the parameters of the mathematical model of the fermentation process.

Contrary to the constant parameters μ_{m} and P_{i} in Eqs. (1)–(3) are $\mu_{\mathrm{m}\vartheta}(t)$, $P_{\mathrm{i}\vartheta}(t)$ functions, which depend on the bioreactor's temperature. Function $\mu_{\mathrm{m}\vartheta}(t)$ denotes the impact of temperature on the maximum microorganisms' growth rate (h^{-1}), and function $P_{\mathrm{i}\vartheta}(t)$ denotes the impact of temperature on the product inhibition constant (g/L). Both functions are identified in such a way that they enable good fitting of the mathematical model's responses to the measured trajectories [20]. Different analytical expressions were tested. After their comparison, the following simple functions were chosen, which can assure proper fitting:

$$\mu_{\mathrm{m}\vartheta}(t) = \mu_{\mathrm{m}}\left[1 + k_{\mu\mathrm{m}}[x_4(t) - \vartheta_0]\right] \tag{8}$$

$$P_{\mathrm{i}\vartheta}(t) = P_{\mathrm{i}}[1 + k_{P\mathrm{i}}[x_4(t) - \vartheta_0]] \tag{9}$$

where.

ϑ_0 is the bioreactor's initial temperature (°K),

$k_{\mu\mathrm{m}}$ is the parameter that describes the correlation between the temperature variation and the maximum microorganisms' growth μ_{m} ($^{\circ}\mathrm{K}^{-1}$), and.

k_{Pi} is the parameter that describes the correlation between the temperature variation and the product inhibition constant P_{i} ($^{\circ}\mathrm{K}^{-1}$).

5. Results of the mathematical model

The parameters μ_m, P_i, S_m, S_i, and α of the mathematical model of the fermentation process depending on the used substances and the principle of the operation mode of the batch bioreactor. For the considered laboratory experimental system, the parameters of the mathematical model were obtained from the measured trajectories of the microorganisms, substrate, and product by the particle swarm optimization. The integral absolute error between measured and simulated trajectories represented the applied performance index [20]. The mathematical model parameters, which were obtained from the experiments of the laboratory kefir production and the subsequent optimization, are shown in **Table 1**.

The Eqs. (1)–(3) represent an autonomous mathematical model, that is, the model without input. This is anticipated because the original industrial versions of batch bioreactors do not allow to be influenced during the execution of the fermentation process. Microorganisms and fermentation substrate are inserted into the bioreactor at the beginning of the fermentation process. The time courses of the concentrations of the fermentation process quantities rest only on the used substances. The initial conditions determine the fermentation process dynamics.

The simulation results of the identified mathematical model of the kefir fermentation process in the laboratory batch bioreactor are shown in **Figure 3**. The trajectories show the response of the concentrations of the microorganisms, fermentation substrate, and fermentation product during the fermentation process, which follows the initial states $x_1(0) = 2.6$ g/L, $x_2(0) = 9.0$ g/L and $x_3(0) = 0.1$ g/L. As expected during the fermentation process, the variables that denote microorganisms and product concentration increased, and the substrate concentration variable decreased. The duration of the modeled fermentation process is approx. 10 hours.

For the simulation of the impact of the changing temperature on the trajectories of the quantities of the fermentation process, the model with the Eqs. 4–9 was used. For the studied fermentation process with data in **Table 1**, the following values of the additional coefficients $k_{\mu m}$ and k_{Pi} were estimated to obtain a good fitting of the measured and simulated trajectories:

$$k_{\mu m} = 0.1°K^{-1} \quad k_{Pi} = 0.04°K^{-1} \tag{10}$$

Parameter	Value
Maximum growth rate of the microorganisms	$\mu_m = 0.5\ h^{-1}$
Inhibition constant of the fermentation product	$P_i = 7.0$ g/L
Saturation constant of the fermentation substrate	$S_m = 0.42$ g/L
Inhibition constant of the fermentation substrate	$S_i = 62.15$ g/L
Correlation parameter among yield of Fermentation product and growth of microorganisms	$\alpha = 0.9\ \frac{g/L}{g/L}$
Correlation parameter that denotes the yield of Fermentation product independent of the growth of microorganisms	$\beta = 0.001\ h^{-1}$

Table 1.
Identified parameters of the mathematical model of the kefir fermentation process in the laboratory bioreactor.

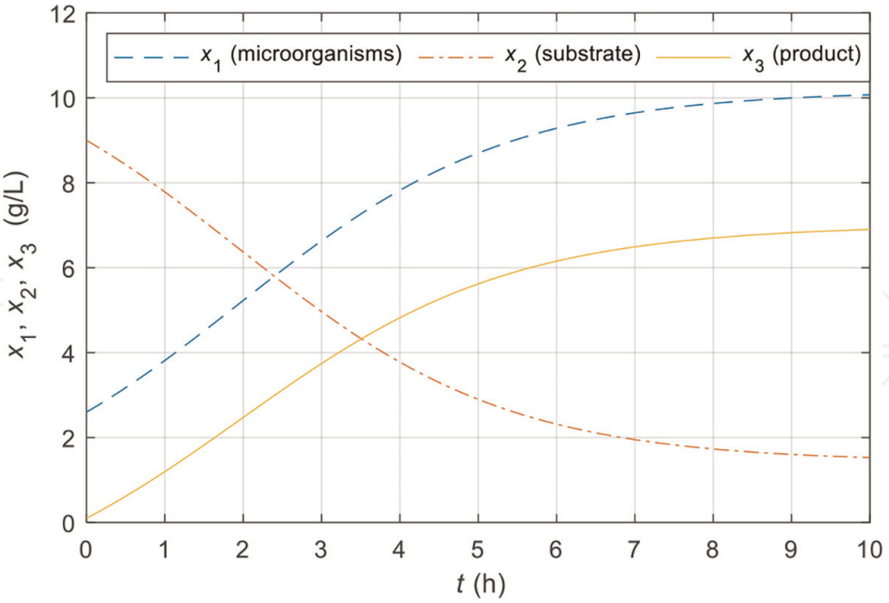

Figure 3.
Simulation results of the milk fermentation process with kefir grains for constant reference temperature. The parameters of the mathematical model are shown in ***Table 1****, presented are concentrations of microorganisms, substrate, and product.*

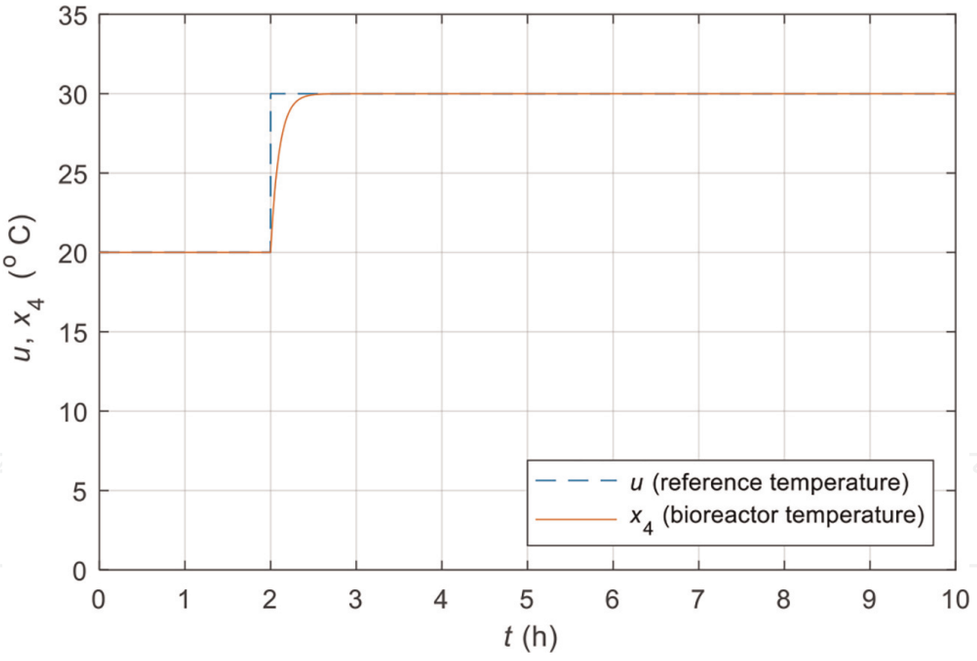

Figure 4.
Simulation results of the milk fermentation process with kefir grains where the step change of the reference temperature at time t = 2 *h is carried out, the parameters of the mathematical model are shown in* ***Table 1*** *and Eq. 10, presented are time responses of the reference temperature of the heating control system, and the actual temperature of the contents of the bioreactor.*

The time constant of the controlled heating system was $T_{\vartheta cs}$ = 0.1 h, and the initial bioreactor's temperature was ϑ_0 = 20°C. The time responses of the reference temperature of the heating control system and the actual temperature of the contents of the bioreactor are seen in **Figure 4**. The step change of the reference temperature at time t = 2 h was generated to produce changes in the time response of the mathematical

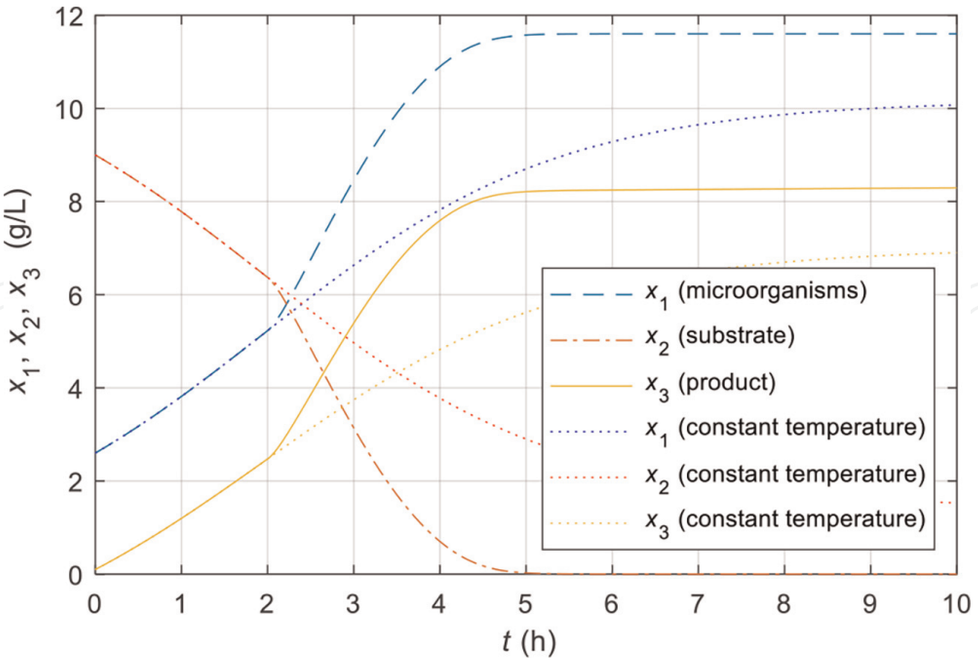

Figure 5.
Simulation results of the milk fermentation process with kefir grains where the step change of the reference temperature at time t = 2 *h is carried out, the parameters of the mathematical model are shown in* ***Table 1*** *and Eq. 10, presented are time responses of concentrations of microorganisms, substrate, and product, dotted/dashed lines show the responses without temperature change.*

model of the fermentation process (4)-(9). The time responses that show the growth of the microorganisms, the consumption of the substrate, and the rising of the fermentation product are shown in **Figure 5**. It is evident that temperature changes generate substantial variations in the dynamics and also in the steady state of the fermentation process. The dotted lines in **Figure 5** represent the time responses of the microorganisms, substrate, and product when no temperature change is made (the same trajectories as in **Figure 1**).

Based on simulations and laboratory tests [18], it was confirmed that changing the reference temperature of the media in the bioreactors significantly impacts the transients and steady state of the fermentation process. This finding confirmed the idea that the bioreactor's heating system could be used to control the fermentation process.

6. Control systems for fermentation process in batch bioreactors

6.1 Conventional control system with a linear controller

The batch bioreactor, which is equipped with a heating system with the possibility of changing the temperature of the medium and with a measuring system for measuring the concentration of the dissolved CO_2, enables the implementation of a conventional feedback control structure. Although the controlled plant is nonlinear and the analysis of its linearized model shows that the variations of parameters of the linearized model are considerable, it was founded that it is possible to use a linear controller with fixed parameters. In this case, the controller must be selected to ensure the stability of the control system in the entire range. The controller chosen in this way will ensure the stability of the control system in the whole operation range. By

selecting the performance index and using the optimization method, we can ensure that the controller will provide optimum performance in the broadest possible operating range.

The main disadvantage of this approach is the need for knowledge of the most accurate mathematical model of the fermentation process of the batch bioreactor. Development of an accurate enhanced mathematical model is very complex and requires a lot of time. First of all, the fermentation process at a fixed (unchangeable) temperature must be carried out. The trajectories of the concentrations of microorganisms, substrate, and the product must be measured and nonlinear model parameters (μ_m, P_i, S_m, S_i, α, β) must be calculated. Then, the fermentation with the same initial concentrations must be rerun, this time with the difference that the temperature of the bioreactor mixture is not constant all the time, but that a step change of temperature occurs during fermentation.

6.2 Adaptive control system

6.2.1 Survey of adaptive control systems

The main difficulties in the use of conventional control systems are incomprehension and uncertainty of the mathematical model of the batch bioreactor and, in the case when using a linear controller, changing the parameters of the linearized model of the fermentation process during the operation. The theory of adaptive systems serves as an ideal foundation for developing a control system for batch bioreactors. Adaptive control systems are applicable in case of unknown and changing structures and parameters of the controlled plants and consequently their mathematical models. Adaptive controllers are capable to adapt parameters to the controlled plant variations.

In publications in the field of Systems Theory, it is possible to trace an enormous number of different adaptive and semi-adaptive theories useful for the development of adaptive control systems. Different adaptive approaches are suitable for different controlled plants and require more or fewer controlled plants' quantities to be measured. Controlled plants may be linear or nonlinear, may have a known or unknown structure, may have unknown constant parameters, or their parameters may change during operation. Some adaptive approaches require measurements of all state variables; others require measurements of only one or a few output quantities.

Very many (almost all) adaptive control approaches are derived from the underlying assumption that a controlled plant is linear and has unknown and constant parameters. For such a (simple) case, theoretical proof could be given of the global stability of the entire adaptive system. In practice, however, many adaptive control approaches have shown to be useful, even in cases where the controlled plants are nonlinear, and their parameters are changeable. In this case, the adaptation mechanism adjusts the parameters of the adaptive controller or explicitly generates a control input according to the linearized model of the controlled plant at the current operating point.

Two fundamental concepts are applied to the design of adaptive control systems.

The first approach is based on the separate utilization of three modules: An identification module that identifies the mathematical model of the controlled plant; a setting module that calculates the parameters of the controller on the basis of the identified mathematical model, and a controller module that calculates the corresponding control signal by means of the calculated parameters. This approach is

called indirect adaptive control, sometimes also self-tuning control (STC), because initially, it was used primarily for self-tuning of the controller for unknown controlled plants. The benefit of the indirect adaptive control is its modular concept, which enables a connection of various identification techniques (Maximum Likelihood, Least Squares, ...), and different techniques for synthesis of the controller (stochastic, deterministic). The combination of the recursive Least Squares identification method and the pole-shifting controller proved to be one of the most successful. Due to such implementation, the indirect control system is very transparent. During its operation, it is possible to monitor the intermediate results of the adaptive process. The main disadvantage of the indirect adaptive control system, however, is that it is challenging to prove (and ensure) the global stability of the entire adaptive control system. The global stability of the self-tuning adaptive control systems is proven only in the case of more straightforward, less applicable controller synthesis methods [26].

The second approach is based on the use of two components: A reference model and an adaptation mechanism. The reference model prescribes the desired static and dynamic behavior of the controlled plant, and the adaptation mechanism generates control input to ensure that the control plant's output (or/and state variables) follow the output (or/and state variables) of the reference model. This adaptive concept is called direct adaptive control because the input of the controlled plant is determined directly. The other name for these control is also Model Reference Adaptive Control (MRAC). The development of the MRAC approaches is based on assuring the stability of a complete adaptive system. This is the reason that MRAC systems have proven global stability. The majority of direct adaptive control systems can be ranged in one of the following adaptive concepts [27]:

- Model reference adaptive control systems where all controlled plant state space variables must be measured (MRAC with full state access, abbreviated MRAC-FSA) [28],

- Model reference adaptive control systems where only the controlled plant output is measured, and an additional observer is used to observe state space variables (MRAC with an adaptive observer, abbreviated MRAC-AO) [29], and

- Model reference adaptive control systems where only the controlled plant output is measured but no additional observer is needed. In this case, to enable proof of global stability, it is necessary that the controlled plant is "almost" strictly positive real. Therefore, the abbreviation MRAC-ASPR is used [30].

For the needs of the implementation of the batch bioreactor control system, the MRAC-FSA algorithms have proved to be less adequate. Difficulties in applying the MRAC-FSA algorithms are found primarily in the unmeasurability of the necessary state-space variables, which results in the unfulfillment of Erzberger's perfect model following conditions that are required for the implementation of MRAC-FSA control [28].

MRAC-AO algorithms do not require access to all state variables of the controlled plant; the measurement of the output quantity is sufficient. However, when using MRAC-AO systems, accurate knowledge is required of the structure of the mathematical model of the controlled plant. It is necessary to know exactly the maximum order of the mathematical model of the controlled plant (together with the unmodeled dynamics); it is also essential to know the exact relative order of the controlled plant's

mathematical model. A limitation is also the requirement that the controlled plant must be a non-minimum phase. All these requirements restrain the use of MRAC-AO algorithms for the control of batch bioreactors.

MRAC-ASPR is the newest of these adaptive concepts. It does not require the usage of an adaptive observer or measurement of all controlled plant variables. MRAC-ASPR is simple for implementation and realization due to time undemanding algorithms. Because of many benefits, the MRAC-ASPR concept was applied to batch bioreactor control.

6.2.2 Theory of MRAC-ASPR system

The presented nonlinear model of the fermentation process can be linearized around the trajectory. This makes it possible to implement the MRAC-ASPR theory, which was originally used for the linear systems described by Eqs. (11) and (12):

$$\dot{\boldsymbol{x}}(t) = \boldsymbol{A}(t)\boldsymbol{x}(t) + \boldsymbol{b}(t)u(t) \tag{11}$$

$$y(t) = \boldsymbol{c}^{T}(t)\boldsymbol{x}(t) \tag{12}$$

where the symbols are:
$\boldsymbol{x}(t)$, $u(t)$, $y(t)$: controlled plant state-space vector, input, output, and.
$\boldsymbol{A}(t)$, $\boldsymbol{b}(t)$, $\boldsymbol{c}^{\mathrm{T}}(t)$: controlled plant system matrix, input vector, and output vector.
The elements of the system matrix $\boldsymbol{A}(t)$, the input vector $\boldsymbol{b}(t)$, and the output vector $\boldsymbol{c}^{\mathrm{T}}(t)$ are unknown and changeable. It is assumed that:

- The range of the elements of the controlled plant matrices $\boldsymbol{A}(t)$ and vectors $\boldsymbol{b}(t)$ and $\boldsymbol{c}^{\mathrm{T}}(t)$ is bounded,
- All possible pairs $\boldsymbol{A}(t)$ and $\boldsymbol{b}(t)$ are controllable and output stabilizable,
- All possible pairs $\boldsymbol{A}(t)$ and $\boldsymbol{c}^{\mathrm{T}}(t)$ are observable.

The desired dynamics of the batch bioreactor can be described by Eqs. (13) and (14):

$$\dot{\boldsymbol{x}}_{\mathrm{m}}(t) = \boldsymbol{A}_{\mathrm{m}}\boldsymbol{x}_{\mathrm{m}}(t) + \boldsymbol{b}_{\mathrm{m}}u_{\mathrm{m}}(t) \tag{13}$$

$$y_{\mathrm{m}}(t) = \boldsymbol{c}_{\mathrm{m}}^{\mathrm{T}}\boldsymbol{x}_{\mathrm{m}}(t) \tag{14}$$

where the symbols are:
$\boldsymbol{x}_{\mathrm{m}}(t)$, $u_{\mathrm{m}}(t)$, $y_{\mathrm{m}}(t)$: reference model state-space vector, input, output, and.
$\boldsymbol{A}_{\mathrm{m}}(t)$, $\boldsymbol{b}_{\mathrm{m}}(t)$, $\boldsymbol{c}_{\mathrm{m}}{}^{\mathrm{T}}(t)$: reference model system matrix, input vector, and output vector.
The reference model determines the desired input-output relation. Therefore, it is not necessary for the reference model order to be the same that the controlled plant order—it could be smaller as written in Eq. (15):

$$\dim[x_{\mathrm{m}}(t)] \ll \dim[x(t)] \tag{15}$$

The deviation between the reference model and controlled plant dynamics is described by the error between both outputs:

$$e_y(t) = y_{\mathrm{m}}(t) - y(t) \tag{16}$$

The Lyapunov stability theory is used for the derivation of the adaptive control algorithm (17) [31, 32]. This algorithm calculates such input of the controlled plant $u(t)$ that assures that the output of the controlled plant $y(t)$ will follow the reference model output $y_{\mathrm{m}}(t)$ in case of unknown controlled plant model $\boldsymbol{A}(t)$, $\boldsymbol{b}(t)$, and $\boldsymbol{c}^{\mathrm{T}}(t)$.

$$u_{\mathrm{p}}(t) = K_e(t)e_y(t) + \boldsymbol{K}_x(t)\boldsymbol{x}_{\mathrm{m}}(t) + K_u(t)u_{\mathrm{m}}(t) \tag{17}$$

where the additional symbols are:
$\boldsymbol{K}_x(t)$, $K_u(t)$: control gains, and.
$K_e(t)$: output feedback parameter.
Variables $e_y(t)$, $\boldsymbol{x}_{\mathrm{m}}(t)$, and $u_{\mathrm{m}}(t)$ and parameters $K_e(t)$, $\boldsymbol{K}_x(t)$, and $K_u(t)$ can be rewritten with control variables vector $\boldsymbol{r}(t)$ and adaptive gains vector $\boldsymbol{K}(t)$:

$$\boldsymbol{K}(t) = [K_e(t) \;\; \boldsymbol{K}_x(t) \;\; K_u(t)] \tag{18}$$

$$\boldsymbol{r}^{\mathrm{T}}(t) = \begin{bmatrix} e_y(t) & \boldsymbol{x}_{\mathrm{m}}(t) & u_{\mathrm{m}}(t) \end{bmatrix} \tag{19}$$

The adaptive gains vector $\boldsymbol{K}(t)$ can be calculated with the proportional-integral algorithm:

$$\boldsymbol{K}(t) = \boldsymbol{K}_{\mathrm{p}}(t) + \boldsymbol{K}_{\mathrm{i}}(t) \tag{20}$$

where symbols denote:
$\boldsymbol{K}_{\mathrm{p}}(t)$: proportional term $\boldsymbol{K}_{\mathrm{p}}(t)$, and.
$\boldsymbol{K}_{\mathrm{i}}(t)$: integral term.
The integral term $\boldsymbol{K}_{\mathrm{i}}(t)$ guarantees convergence and the proportional term $\boldsymbol{K}_{\mathrm{p}}(t)$ generates a fast response. The MRAC-ASPR algorithm increases the values of the adaptive gains vector $\boldsymbol{K}(t)$ if the error increase and decreases if the error decrease [28]. Both terms of the adaptive gains vector $\boldsymbol{K}(t)$ can be calculated by Eqs. (21) and (22) [28]:

$$\boldsymbol{K}_{\mathrm{p}}(t) = e_{\mathrm{y}}(t)\boldsymbol{r}^{\mathrm{T}}(t)\,\boldsymbol{T}' \tag{21}$$

$$\dot{\boldsymbol{K}}_{\mathrm{i}}(t) = e_{\mathrm{y}}(t)\boldsymbol{r}^{\mathrm{T}}(t)\,\boldsymbol{T} \tag{22}$$

where symbols denote:
$\boldsymbol{T}'$: positive semi-definite matrix, and $\boldsymbol{T}$: positive definite matrix.
Considering (22) for the calculation of $\boldsymbol{K}_{\mathrm{i}}(t)$, it is obvious that the proposed integral term in the presence of disturbances, whenever perfect following is not possible, may reach unnecessarily large values, or may even diverge. In order to improve the convergence of the adaptive system, the following modification of the integral term was proposed [30]:

$$\dot{\boldsymbol{K}}_{\mathrm{i}}(t) = e_{\mathrm{y}}(t)\boldsymbol{r}^{\mathrm{T}}(t)\,\boldsymbol{T} - \sigma\boldsymbol{K}_{\mathrm{i}}(t) \tag{23}$$

where the σ-term is used to evade integral gains divergence in the presence of a disturbance.

The origin theory for the development of the MRAC-ASPR control systems is described in detail in [30].

7. Results of the control systems

Both considered control strategies were tested for the control of the fermentation process in the batch bioreactor with the data in **Table 1**. The treated batch bioreactor was equipped with a final control system for heating/cooling the bioreactor's contents and with a measurement system that measures the concentration of the dissolved CO_2 in the bioreactor's vessel. The input of the thus-formed controlled plant represents the reference signal for the heating/cooling system, and the output of the controlled plant is the signal that corresponds to the dissolved CO_2 concentration.

7.1 Reference trajectory of the dissolved CO_2

The quantity and quality of the product in the batch bioreactor are decisively dependent on the trajectories of the biological quantities in the fermentation process, affecting the kinetics of the bioprocess [1, 3, 12, 22]. In today's used industrial batch bioreactors without a closed-loop control system, the course of the fermentation process depends only on the substances that were placed in the bioreactor before the beginning of the fermentation process and cannot be influenced during the fermentation process. The developed and presented control system makes the changing of the time responses of the biological quantities possible.

With this new possibility, the presented control system will enable the fermentation process to be carried out, where the time courses of concentrations of biomass, substrate and fermentation result will be the same as the prescribed reference trajectories. It makes sense that the reference trajectories are chosen in such a way that the fermentation process gives as much as possible and as much quality product as possible in the shortest possible time. There are many publications that describe in more detail how to select reference trajectories [12]. In the presented contribution, we did not theoretically deal with the problem of choosing reference trajectories but limited ourselves only to choosing the reference trajectory of the concentration of dissolved CO_2. The reference trajectory was chosen based on the experience of operators operating batch bioreactors.

The basis for the selection of the reference signal is the time course of the corresponding quantity of the unregulated fermentation process. When choosing a reference trajectory, we must take into account the biological limitations of the fermentation process. The dynamics of the reference signal must be such that the regulation system of the bioreactor can realize it. For the tested fermentation process in the available bioreactor, the following reference trajectory $r(t)$ for the dissolved CO_2 was chosen:

$$r(t) = 0.8\left(1 - e^{-t/1.5}\right) \tag{24}$$

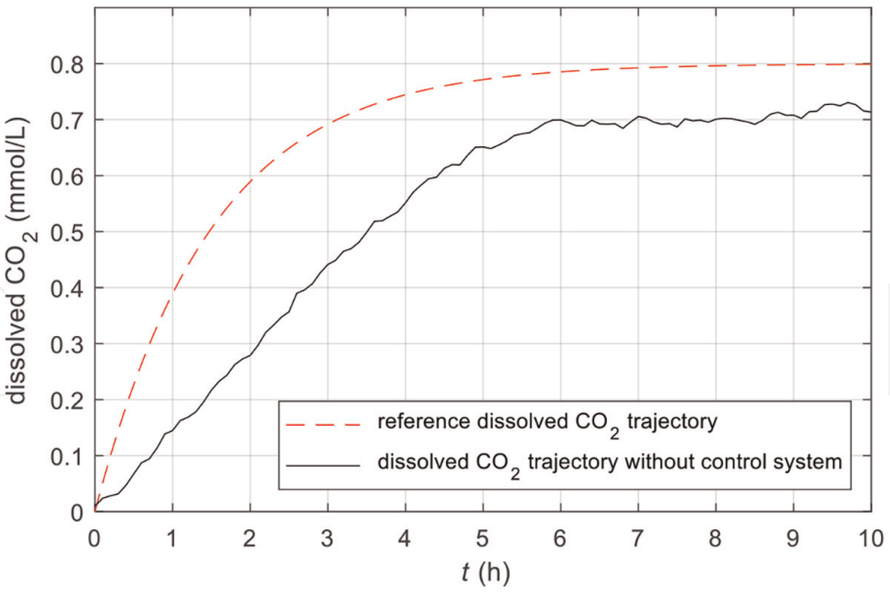

Figure 6.
Actual and the reference trajectory of the CO_2 concentration; data of the bioreactor are in ***Table 1****.*

The time response of the CO_2 concentration of the unregulated bioreactor and the chosen suitable reference trajectory for the control system is shown in **Figure 6**.

7.2 Conventional control system with a linear controller

The use of linear controllers makes sense in the case of a batch bioreactor since the analysis of a linearized mathematical model in the vicinity of the trajectory showed a relatively small range of variations in the model's parameters. In such cases, the controller must be set to ensure stability and good transients of the control system in the most problematic operating range. Of course, the main limitation in the use of such controllers is the requirement to identify the mathematical models of the controlled plants.

A simple PI controller with transfer function $G_{\mathrm{PI}}(s)$ [33] is used to demonstrate the usefulness and efficiency of the conventional control system with a linear controller for the batch bioreactor control.

$$G_{\mathrm{PI}}(s) = k_{\mathrm{p}} \frac{sT_{\mathrm{i}} + 1}{sT_{\mathrm{i}}} \tag{25}$$

where k_{p} is the gain and T_{i} is the time constant of the PI controller described with the transfer function $G_{\mathrm{PI}}(s)$.

The controller design is based on the known nonlinear and linearized mathematical models. The design procedure was divided into two phases:

- First, the time constant T_{i} of an integral part of the PI controller was determined regarding the unstable region of the operating range. The selected time constant was determined on the basis of the linearized mathematical model, and assures a stable operation for small deviations around the entire trajectory.

- Second, the gain k_p of the proportional part of the PI controller was calculated using the differential evolution. Integral time square cost function J of the output error variable and input variable (26) was used to determine the optimal value of the controller's gain [21].

$$J = \int_0^{t_f} \left\{ Q\,[y^*(t) - y(t)]^2 + R[u^*(t) - u(t)]^2 \right\} dt \tag{26}$$

where.

$u(t)$ is the input variable of the controlled plant (i.e., the reference temperature of the bioreactor's temperature control system (°K)),

$y(t)$ is the output variable of the controlled plant (i.e., the output of the measurement system for the dissolved CO_2 concentration (mmol/L)) mathematical model,

$u^*(t), y^*(t)$ are the nominal input and output variables of the controlled plant,

Q, R are the weighting parameters of the quadratic cost function, and.

t_f - final time.

In the optimization calculations, it is assumed that the nominal input variable is constant during the entire fermentation process and is equal to the temperature of the bioreactor's filling at the beginning of the fermentation process ϑ_0 (°K). For the chosen cost function's parameters Q, R, t_f, and the constant temperature of the bioreactor's filling ϑ_0:

$$Q = 1\, R = 0.1\, t_f = 10\, h \quad \vartheta_0 = 292°\text{K} \tag{27}$$

the following parameters of the PI controller were calculated:

$$k_p = 22.0\ T_{PI} = 1.5\ \text{h} \tag{28}$$

The corresponding block diagram of the control system is shown in **Figure 7**. The obtained results are presented in **Figures 8** and **9**. The time response of the actual

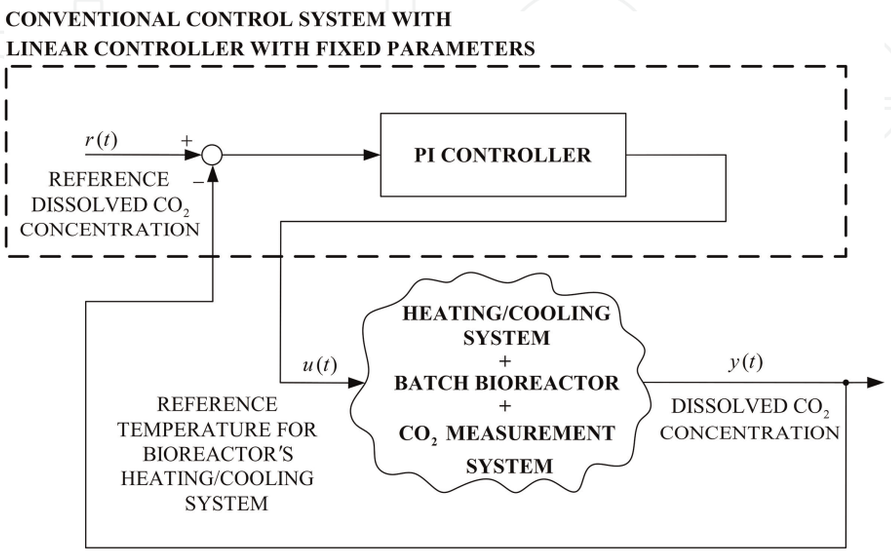

Figure 7.
Block diagram of the conventional control system with linear controller.

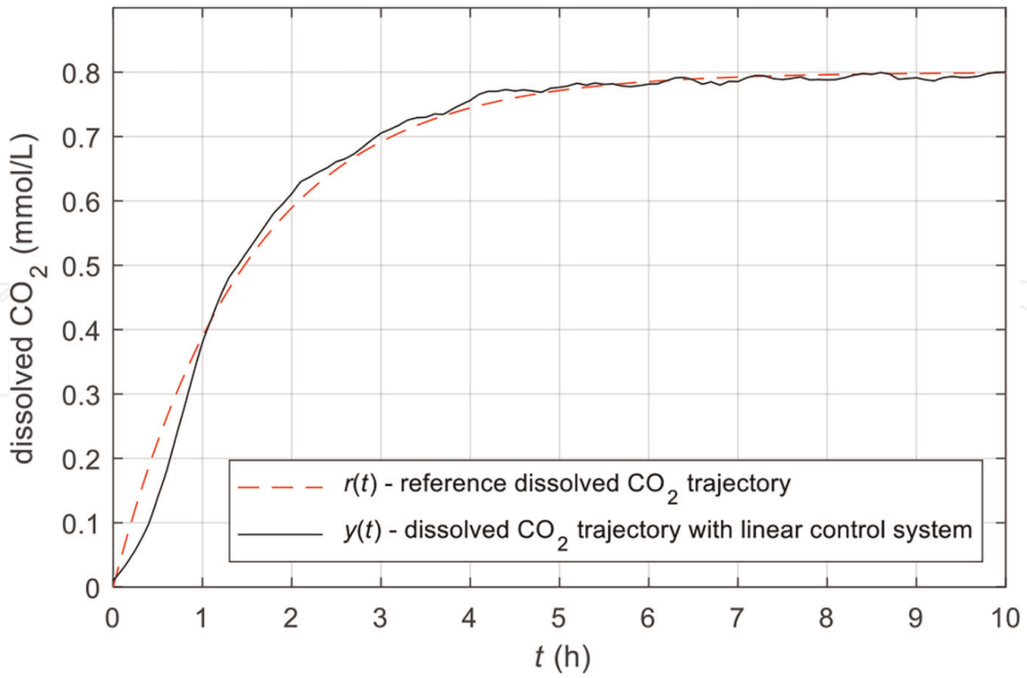

Figure 8.
Time response of the actual and reference-dissolved CO_2 concentration of a batch bioreactor with the conventional control system with a linear controller with calculated parameters; data of the bioreactor are in ***Table 1****, controller parameters in ref. (28).*

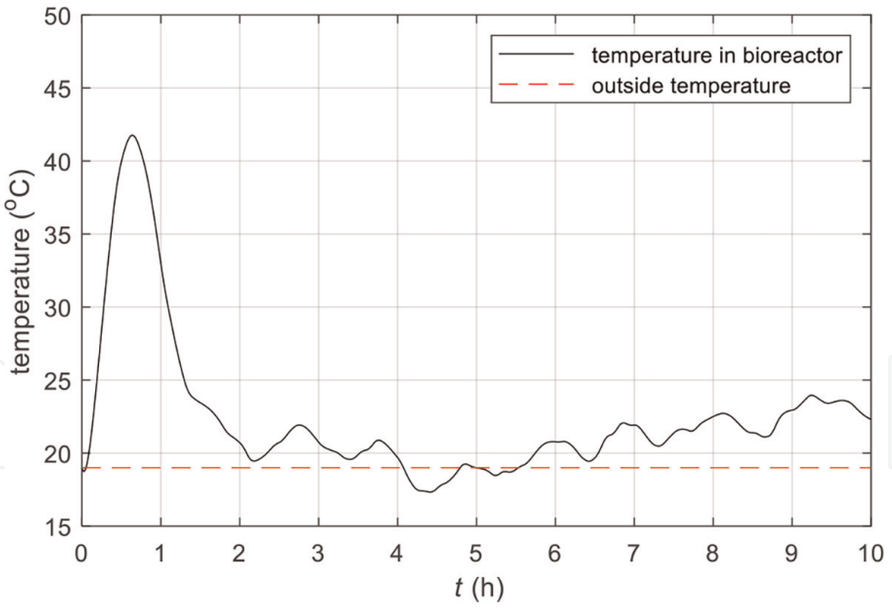

Figure 9.
Time response of the batch bioreactor's inner temperature and the constant outside temperature when the conventional control system with a linear controller with calculated parameters was used; data of the bioreactor are in ***Table 1****, controller parameters in ref. (28).*

dissolved CO_2 concentration of a batch bioreactor controlled with a conventional PI controller, together with the reference trajectory, is shown in **Figure 8**. The bioreactor's inner temperature $x_4(t)$, as a consequence of the control of the heating/cooling system and the outside temperature ϑ_0 are presented in **Figure 9**.

7.3 Adaptive control system

A simple adaptive control system based on the MRAC-ASPR control theory was used for the batch bioreactor's control implementation. The block diagram of the adaptive control system for the batch bioreactor's control is shown in **Figure 10**.

The proposed adaptive control system represents a kind of model reference adaptive control system—the adaptation mechanism and control law will ensure that even in the case of unknown and changeable parameters of the controlled plant, the course of the fermentation process will be as close as possible with the dynamics of the selected reference model. The reference model was determined based on the selected reference signal. For the selected reference signal (24), we chose 1st order lag with gain $k_{rm} = 0.8$ and the time constant $T_{rm} = 1.5$ h as the reference model.

The speed of the adaptation mechanism depends on the matrix parameters $\boldsymbol{T'}$ and $\boldsymbol{T}$. To ensure the stability of the adaptive system, it is required that the matrix $\boldsymbol{T'}$ is

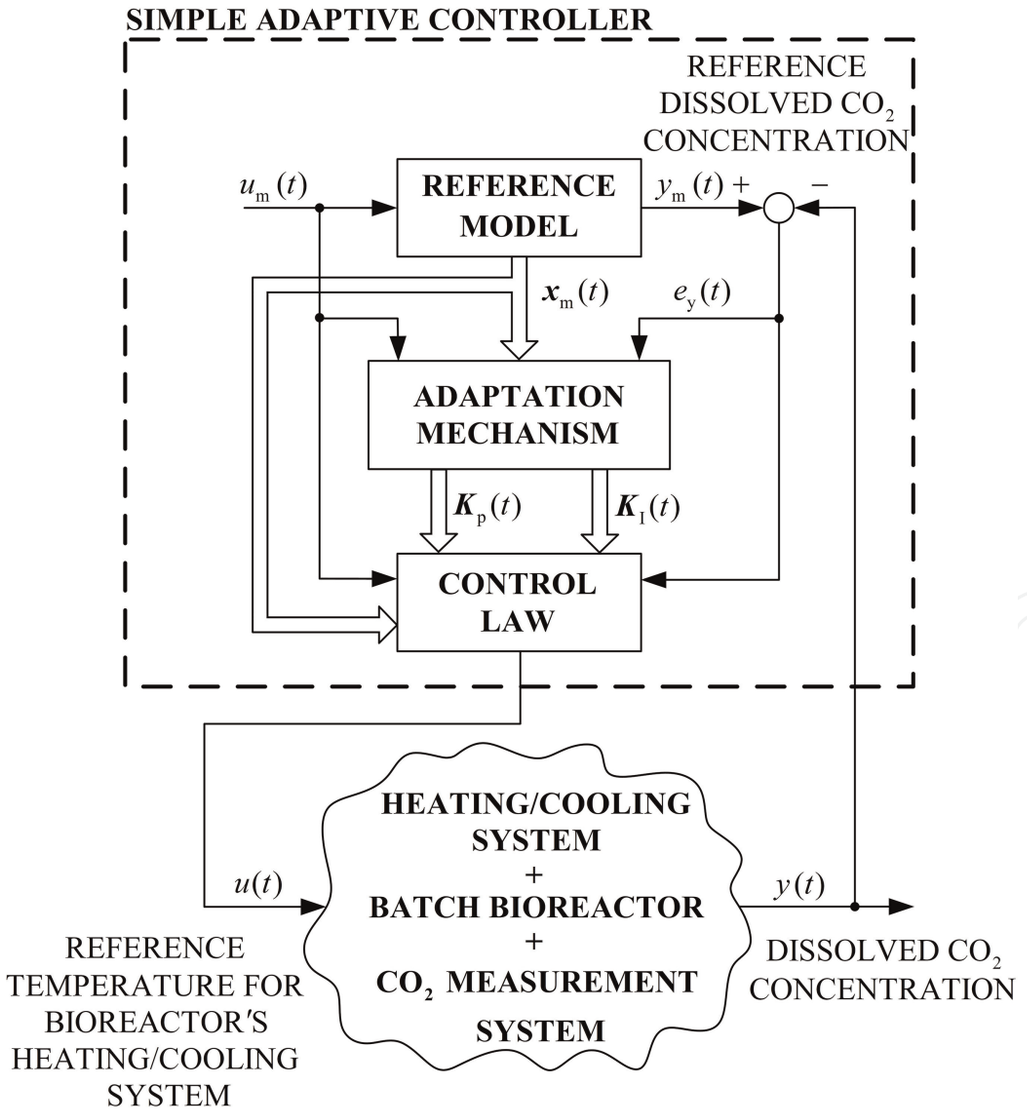

Figure 10.
Block diagram of the adaptive control system.

positive semi-definite and the matrix $\boldsymbol{T}$ is a positive definite matrix. Numerical simulations were used to choose the parameters:

$$\boldsymbol{T} = 4 \cdot 10^3 \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \boldsymbol{T}' = 4 \cdot 10^3 \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{29}$$

To avoid divergence of an integral part in the presence of disturbance the following σ-term was used:

$$\sigma = 0.95 \tag{30}$$

The results of the simple adaptive control technique for the fermentation process in the batch bioreactor are shown in **Figures 11** and **12**. **Figure 11** shows the reference and the actual time response of the dissolved CO_2 concentration when a simple adaptive controller was used. The obtained results show that with the proposed adaptive regulation system, we can achieve the time course of the dissolved CO_2 concentration will be very similar (almost the same) as the prescribed reference time course. The regulation system ensures the same course of the fermentation process even in the case of small variations in the initial values of the quantity and quality of the fermentation components (different charges).

The control system ensures the same dynamics of the fermentation process by changing the temperature in the bioreactor. **Figure 12** shows the required temperature in the bioreactor. It can be seen from **Figure 12** that the temperature required for the control remains within an acceptable temperature range.

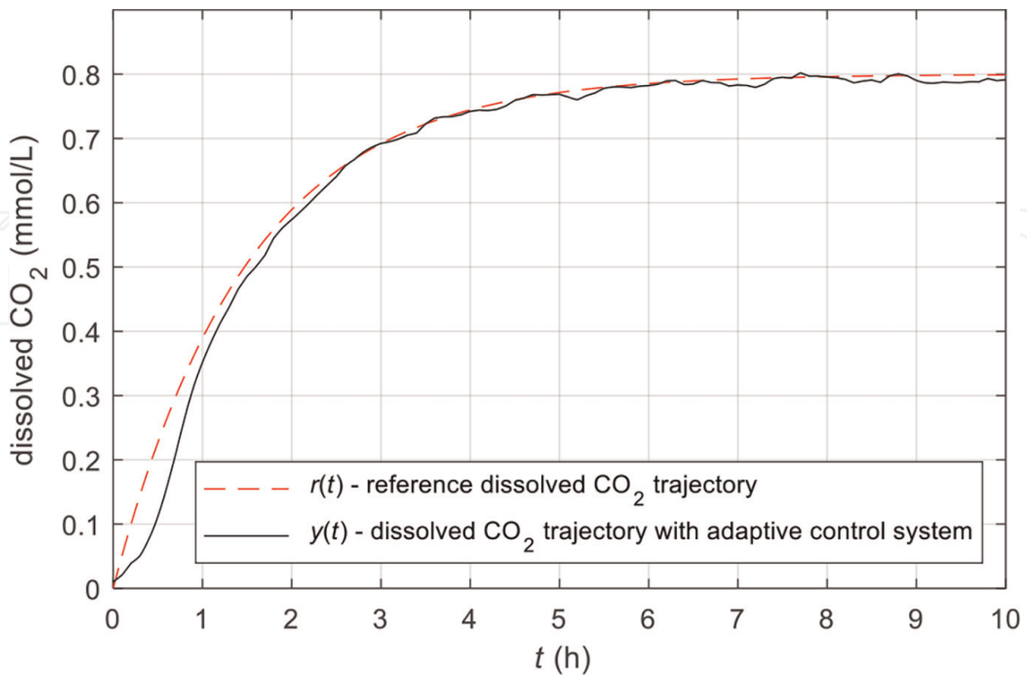

Figure 11.
Time response of the actual and reference dissolved CO_2 concentration of a batch bioreactor with the simple adaptive control system; the data of the bioreactor are shown in ***Table 1****, and adaptation mechanism parameters in [29, 30].*

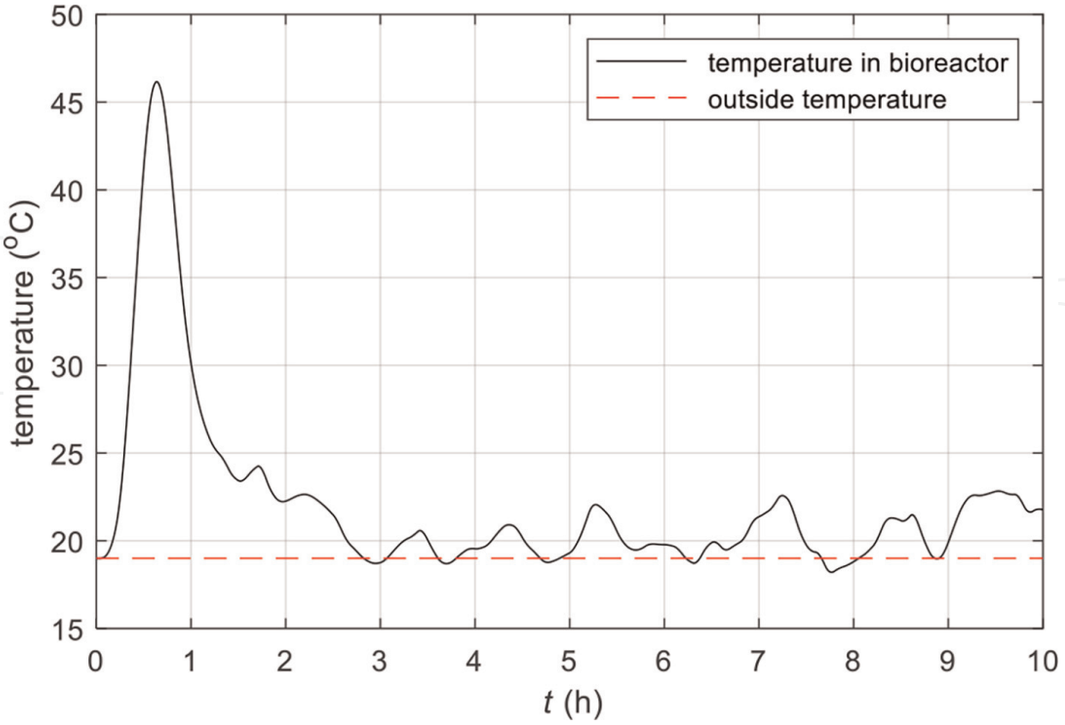

Figure 12.
Time response of the batch bioreactor's inner temperature and the constant outside temperature when the simple adaptive control was used; the data of bioreactor are shown in ***Table 1****, and adaptation mechanism parameters in [29, 30].*

8. Discussion

At first sight, the results obtained with both presented control systems are excellent and very similar. Almost identical dynamics of the fermentation process were obtained, as defined by the reference trajectory. To achieve these responses, acceptable changes were requested in the bioreactor's inner temperature.

To obtain better insight into the performances of the control systems, the evaluation based on the performance index is meaningful. The integral quadratic performance index, the same as the cost function shown in Ref. (26), was used for the comparison. The same parameters of the performance index as for the PI-controller optimization were used to estimate control quality. The calculated performance indexes for the conventional control system with PI controller and the simple adaptive control system are presented in **Table 2**.

It is surprising that the performance indexes of conventional PI control system and advanced adaptive control system are very similar. From the point of view of the quality of the control systems, there is no significant difference between the PI control system and the adaptive control system. However, there is a significant difference in

Control Concept	Value
Conventional control system with PI controller	J = 4.2496
Simple adaptive control system	J = 4.2864

Table 2.
Performance indexes for the studied Bioreactor's control systems.

the use of both control systems. Using the PI control system is very time-consuming for bioreactor operators. To determine the optimal parameters of the PI controller, a precise determination of the mathematical model of the fermentation process is necessary. This is very demanding and time-consuming. The adaptive controller has a great advantage, as it automatically adapts to the controlled system. Operators do not need to set the parameters of the controller, which significantly simplifies and speeds up their work.

Despite the slightly worse performance index, the proposed adaptive control approach presents a much better choice for the development of the practical control system for the batch bioreactor.

9. Conclusion

There are two important contributions to the presented work:

- It has been shown that batch bioreactors, which represent the simplest and cheapest type of bioreactors and which basically allow only autonomous (uncontrolled) operation, can be turned into a closed-loop control system with a little additional equipment. The basic condition is that the bioreactors have a heating/cooling system, through which the controller can influence the course of the fermentation process, and

- It has been shown that the developed adaptive control system represents a very effective control for batch bioreactor operation, even in the case of an unknown, nonlinear, and time-variable mathematical model of the fermentation process.

The chapter shows the possibility of using two different control concepts: conventional PI control system and advanced adaptive control system. The adaptive control gives almost the same results as the PI control system, whose parameters were offline tuned with the optimization, on the basis of the identified mathematical model of the fermentation process.

Adaptive control system proved to be significantly more suitable for the control of batch bioreactors. Its main advantage is very simple to use, which does not require a prior setting of parameters, but the regulator itself adapts to the characteristics of the controlled fermentation process. In such a way, the batch bioreactors, which are easy to fabricate and maintain (and are, therefore, consequently less flexible to control), thanks to the advanced control theory, easily and cheaply acquire the possibility to improve their performance significantly. The shorter fermentation time and higher quality of the obtained products are guaranteed, which is reflected in their greater efficiency of operation.

In the future, we plan to continue the work we have started. The goal is to simplify the realization of the control system and to carry out testing on as many different batch bioreactors as possible.

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