MANTAS BUTKUS

DEVELOPMENT AND INVESTIGATION OF ADAPTIVE CONTROL ALGORITHMS FOR BIOTECHNOLOGICAL PROCESSES

DOCTORAL DISSERTATION

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Technological Sciences, Informatics Engineering (T 007)

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Contents

List of terms and abbreviations

AGSMC	Adaptive gain sliding mode strategy
ANN	Artificial neural network
CDC	Carbon dioxide concentration
CER	Carbon dioxide evolution rate
CPR	Carbon production rate
DEE	Differential equation editor
DO	Dissolved oxygen
DOC	Dissolved oxygen concentration
E.coli	Escherichia coli
FIS	Fuzzy interference system
FOPTD	First order plus time delay
FKBC	Fuzzy knowledge based control
GA	Genetic algorithm
GS	Gain scheduling
IAE	Integral absolute error
IEEE	Institute of Electrical and Electronics Engineers
IFNa5	interferon-alpha 5
IMC	Internal model control
ISE	Integral squared error
ITAE	Integral time absolute error
MAE	Mean absolute error
MFA	Model-free adaptive
MRAC	Model reference adaptive control
OUR	Oxygen uptake rate
PAT	Process analytical technology
PD controller	Proportional-derivative controller
PI controller	Proportional-integral controller
PID controller	Proportional-integral-derivative controller
P&ID	Piping and instrumentation diagram
RMSE	Root mean squire error
R&D	Research and Development
SGR	Specific growth rate
UML	Unified Modeling Language

INTRODUCTION

Relevance of the research

The last few decades have seen the growing application of biotechnological processes in the industry [1, 2], which is explained by the improvement of profitability and quality in industrial production, new legislative standards in industrial technologies, as well as some other reasons. Adaptive control algorithms have a major impact on the technological progress and efficiency. Over the last two decades, considerable efforts have been made to design and develop innovative adaptive control algorithms. The academic community has proposed various adaptive control methods; however, they tend to be complex, and require a lot of time and knowledge to develop and fine-tune. The most well-known works are of the researchers K. Åström, B. Wittenmark and M.M. Gupta, who have worked extensively in this field. However the challenges of developing adaptive control algorithms operating in the unknown, non-linear and time-varying environment inherent in the biotechnological processes have not yet been adequately addressed from the theoretical and practical points of view, therefore, adaptive control algorithms are still rarely used to control biotechnological processes in the world's leading biotechnology companies. Therefore, simple and easy to implement adaptive control techniques are needed for successful implementation in the industry.

Scientific-technological problem and working hypothesis

Biotechnological processes are among the most complex control objects with all the characteristics that make the control difficult:

1. Non-linear relationships between process variables.
2. Time-varying dynamic properties.
3. Lack of sensors that can provide reliable process monitoring.

Therefore, the development of efficient control algorithms is a relevant task for bioengineering. The need for adaptive control algorithms is high, and they are needed to develop new and improve the already available biotechnological processes in both scientific laboratories and industry. The academic community has proposed various adaptive control methods; however, they tend to be complex, and they also require a lot of time and knowledge to develop and fine-tune. Therefore, simple and easy to implement adaptive control techniques are needed for successful implementation in the industry.

Research object

The research concentrates on the development and investigation of algorithms for adaptive biotechnological process control.

The aim of the research

This doctoral thesis aims to develop adaptive control algorithms for typical biotechnological processes that could be easily implemented in standard industrial controllers.

The objectives of the research

1. Development and study of algorithms for direct and indirect adaptive control of biotechnological processes.
2. Development and study of adaptive control algorithms for biotechnological processes based on fuzzy logic, gain scheduling and statistical analysis.
3. Application of adaptive control algorithms for batch and fed-batch fermentation processes controlling key process parameters (SGR, DOC, pH).
4. Testing and experimentation of control algorithms to manage batch and fed-batch biotechnological processes.

Scientific novelty

Five easy-to-implement adaptive control techniques have been developed and are presented in this doctoral thesis:

- fuzzy logic-based PI controller parameter adaptation;
- gain scheduling-based PI controller parameter adaptation;
- statistical analysis-based PI controller parameter adaptation;
- polynomial-based PI controller parameter adaptation;
- substrate feeding profile adaptation.

Fuzzy logic-based models tend to rely on significant amounts of complex rules and membership functions, thus making these models hard to fine-tune and develop. The proposed fuzzy logic-based adaptive control algorithm adapts the PI controller tuning parameters by using a fixed fuzzy model which consists of only 4 rules and 2 membership functions for one input and two output variables. Process operators can usually provide the required expert knowledge. This minimizes the complexity of the model, thus making it suitable for implementation in fed-batch biotechnological processes.

Gain scheduling-based models tend to rely on external variables that need to be measured to adapt the controller tuning parameters. The developed gain scheduling-based control algorithms use only the controller input/output signals to adapt the controller tuning parameters thus not requiring any additional soft-sensor measurements for the controller development and implementation in batch and fed-batch biotechnological processes.

Adaptive control algorithms tend to rely on complex operations. The developed controller feedback signal statistical analysis and polynomial based adaptive control algorithms are based on basic mathematical operations making the presented algorithms suitable for practical implementation in industrial controllers.

The substrate feeding adaptation model focuses on generating an efficient substrate feeding strategy that ensures controllability of the process and secures sufficient bioprocess productivity. The developed substrate feeding adaptation model is based on entirely experimental data, so it can be easily adapted by bioprocess operators who work with real industrial processes and do not have specialized knowledge in the fields of mathematical modeling and control.

The main advantages of the developed and presented adaptation techniques can be summarized as:

- a simple model structure which relies on the process operator's level of knowledge and basic mathematical operations;
- usage of only controller input/output signals for controller tuning parameter adaptation;
- minimization of the required soft-sensor measurements for the realization of controller tuning parameter adaptation.

To evaluate the performance of the developed systems, the proposed models were compared with standard PI controllers with fixed parameters or similar adaptive control techniques.

Practical significance

1. The developed adaptive control algorithms can be used in the following biotechnology companies in Lithuania:
   (a) *Northway Biotech*, UAB
   (b) *Celltechna*, UAB
2. The methods provided in this thesis have been developed in support of the European Regional Development Fund according to the supported activity *Research Projects Implemented by World-class Researcher Groups* under Measure No. 01.2.2-LMT-K-718.
3. One of the developed methods was patented in Lithuania together with 7 other colleagues from Kaunas University of Technology: Galvanauskas, Vytautas

(autorius, išradimo); Simutis, Rimvydas (autorius, išradimo); Levišauskas, Donatas (autorius, išradimo); Urniežius, Renaldas (autorius, išradimo); Vaitkus, Vygandas (autorius, išradimo); Tekorius, Tomas (autorius, išradimo); **Butkus, Mantas** (autorius, išradimo); Survyla, Arnas (autorius, išradimo). Maitinimo profilių, skirtų valdyti pusiau-periodinius rekombinantinių E. coli kultivavimo procesus, formavimo ir adaptavimo būdas / išradėjai: V. Galvanauskas, R. Simutis, D. Levišauskas, R. Urniežius, V. Vaitkus, T. Tekorius, **M. Butkus**, A. Survyla; savininkas: Kauno technologijos universitetas. LT 6861 B. 2021-11-10.

Approval of the results

The doctoral thesis relies on 2 main papers published in international scientific journals referred to in the *Clarivate Web of Science* database, while, in total, the results have been published in 3 scientific papers. The essential results have been presented in 3 conferences, including the worldwide recognized IEEE 17^{th} International Conference on Automation Science and Engineering. The method presented in Section 2.7 was developed and patented together with 7 other co-authors from Kaunas University of Technology.

The statements presented for defence

1. Fuzzy logic-based adaptive control algorithm development does not require deep process knowledge and relies on expert knowledge that is attainable in the form of qualitative understanding of the relationships between various important process variables. Such knowledge is usually available from the process operators. The developed fuzzy logic-based adaptive control algorithm outperforms other adaptive techniques by compensating deviations that could be caused by various process disturbances or equipment malfunctions, thereby making this algorithm suitable for biotechnological process control.

2. Adaptive control algorithms based on the gain scheduling method, when using only controller input/output variables for parameter adaptation, are suitable for batch and fed-batch bio-technological process control. The developed gain scheduling-based algorithm outperforms the conventional PID controllers with fixed parameters under extreme operating conditions at setpoint tracking (changes across a wide range) and disturbance rejection (acting disturbances of the maximal magnitude). By using only the controller input/output variables, no additional online measurements of the process variables are needed to develop the algorithm for tuning parameter adaptation. This minimizes the equipment for controller implementation.

3. The developed adaptive control algorithms which are based on feedback signal statistical analysis and polynomials are suitable for biotechnological process

control. These algorithms can be applied for pH and DOC control at steady setpoint control in fed-batch fermentation processes. The controller tuning parameters are calculated through simple mathematical operations, which simplifies the implementation in industrial controllers.

4. Glucose accumulation in fed-batch cultivation processes can be avoided by measuring the OUR and the substrate feeding rate-based indicator (the ratio of the amount of oxygen consumed to the amount of glucose supplied to the bioreactor during a selected cultivation time interval) that is used in limited growth cultivation processes to adapt the substrate feeding profile during the cultivation process.

Structure of doctoral thesis

The doctoral thesis is organized as follows. Section 1 is designated for the analysis of the relevant scientific literature with respect to the adaptive control of biotechnological processes and presents an overview of the currently available technologies for adaptive control in biotechnological processes. Section 2 describes the proposed methods for adaptive biotechnological process control. Section 3 presents the results obtained from the investigation of the developed control algorithms. In the same section, a performance evaluation is provided for each developed control algorithm. The doctoral thesis is finished with general conclusions provided in Section 4.

Part of Sections 2–4 has been quoted verbatim from the following sources: [3, 4, 5, 6].

The thesis consists of 142 pages, 82 figures, 28 tables, and 126 references.

Work done in collaboration

The patented substrate feeding adaptation algorithm has been developed in collaboration with 7 other researchers from Kaunas University of Technology. The experiments were conducted in the automation departments R&D laboratory with the help of Renaldas Urniežius team. My main responsibilities were:

1. Virtual bioreactor modeling to test the stability of the controlled algorithm while compensating for disturbances;

2. Participation in experimental test runs in the R&D laboratory;

3. Participation in the result analysis with the research group.

Based on the achieved results, the developed substrate feeding adaptation algorithm has been patented in Lithuania (LT 6861 B) and is currently submitted for a European Patent (EP4083185 (A1)).

1. ADAPTIVE CONTROL OF BIOTECHNOLOGICAL PROCESSES

1.1. Introduction to biotechnological process control

Various challenges and problems nowadays cannot be solved without biotechnology. Medical, pharmaceutical, environmental and many other industries rely heavily on the products created by bioengineers. To highlight the needs and importance of the bioprocess control, particularly for the manufacturing of expensive and/or large-scale products, new process development is one of the fields to look at [7]. Environmental conditions that would be best suited for the growth and production of the target product need to be determined by a bioengineer who would find or genetically modify a potential strain. The control of temperature, pH, DOC, the specific growth rate and some other parameters are usually involved at this stage. Moreover, the concentration regions for the nutrients, precursors, and the so-called trace elements are specified. Whereas, for the former variables, often 'optimal' setpoints are provided which, at least in smaller scale reactors, can - to some extent - be maintained by independent classically designed controllers, the information about the best nutrient supply is incomplete from the control engineering point of view. It is this dynamic nutrient supply which is most often not revealed in the biological laboratory and which, however, offers substantial room for production improvements by control [8].

Whenever bacteria like *E.coli*, yeast, fungi, or any animal cells are used for production, these cells are composed of thousands of different compounds that react with each other in hundreds or more reactions. All reactions are tightly regulated on a molecular and genetic basis. Under unlimited growth conditions, all cellular compartments are built up at the same specific growth rate, which means that the cellular composition does not change over time [9]. In a mathematical model describing growth and production, only one state variable is needed to describe the biotic phase. This leads to unstructured models. Whenever a cell enters a constraint, which is often required for production, the cell begins to restructure its internal response pathways [8]. Unstructured model-based approaches for monitoring and control are now set to fail. The number of biotic state variables needs to be increased. However, it is not clear how this should be done in the most effective manner. Therefore, modelling of the boundary behavior is complicated and essential for the control of biotechnological processes. Huge amounts of process-specific information are required. In addition, model-based estimates of the state of the cell and the environment are very important, since real-time measurements of the cells' internal processes concentrations of nutrients are usually impossible. Finally, the robustness of the methods must be considered, as the models used for process control must be limited in size and thus only provide an approximate description.

Such physical parameters as temperature, pH, dissolved oxygen, or CDC are usually controlled with fixed setpoint tracking algorithms. The most noticeable example could be the biomass growth rate control. Its goal is to achieve a high cell concentration in the reactor as rapidly as possible. In such processes as the cultivation of baker's yeast, or where the maximal amount of the product is the target, this is the predominant goal. For other products not associated with growth, a high cell mass is also preferable because the amount of cells correlates directly with productivity. The usage of unstructured models for model-based control requires unlimited growth that can be achieved by maintaining the feeding profile above a predefined level. However, excess nutrients must be avoided because some organisms, such as baker's yeast, begin excess metabolism with materials that can be inhibiting at later phases of the cultivation. What concerns some products, like, for example, the antibiotic penicillin, the organism must grow gradually to achieve a high production rate [10]. For these so-called secondary metabolites, low but not vanishing concentrations are needed for some limiting substrates. If set points for these concentrations are specified instead, this can present a rather difficult control problem. Since organisms are trying to grow exponentially, it must be possible to increase the controller output exponentially as well. The challenge originates primarily from the imprecise and infrequent measurements with which the soft-sensors or controllers must operate, and from the danger that an intermediate under- or over-supply of nutrients will cause the metabolism to enter an undesirable state of productivity. The development of control algorithms is greatly affected by the number of nonlinearities existing within the process. Classical controllers, such as PID or PI, are sufficient if the confronted nonlinearity is very small. When encountering a significant number of nonlinearities, such linear models are ineffective, mainly due to the fact that even small disturbances can drive the process away from the operating point [11]. Control quality is influenced by the controller's ability to provide a stable performance while dealing with process variability and disturbances [12, 13, 14]. Accurate control of technological parameters during microorganism cultivation processes is necessary for retaining currency with the desired technological regimes and the reproducibility of processes. However, the dynamical parameters of batch and fed-batch cultivation processes vary widely over the cultivation cycle. Therefore, conventional control systems with fixed-gain controllers are not able to provide the required performance [15].

1.2. Adaptive control of biotechnological processes

Adaptive control represents techniques providing a systematic approach for automatic controller parameter adjustments by implementing them close to the real time. This is done to reach or to sustain the required level of control system performance while compensating for the plant model parameter change over time [16]. Firstly, let us analyze the situation where the dynamic model parameters of the controlled plant are unknown but remain constant (at least in a certain region of the operation). Considering these cases, even though the framework of the controller does not get influenced overall by the individual values of the plant model parameters, the appropriate tuning of the controller parameters cannot be done without knowing their values. Adaptive control techniques are able to automatically tune the controller parameters in a closed loop. This leads to the vanishing of the effect created by the adaptation over time. The adaptation procedure may require to restart the controller if the operation conditions change over time.

Now let us analyze the situation when the dynamic model parameters change unpredictably in time. These cases occur due to some environmental condition change or because oversimplified linear models for non-linear systems were used. These situations can also be caused by slowly time-varying parameters of the controlled system. Standard control systems are not able to cope with these changes and to ensure a reasonable level of control system performance therefore, an adaptive control approach has to be taken into account. In such cases, the adaptation will be active most of the time and can be considered non-vanishing for this type of operation. A more detailed explanation how an adaptive control system operates is possible by analyzing the development and tuning approach of a controller depicted in Figure 1.1. The dynamic model of the biotechnological process needs to be known to develop and tune a suitable controller [17].

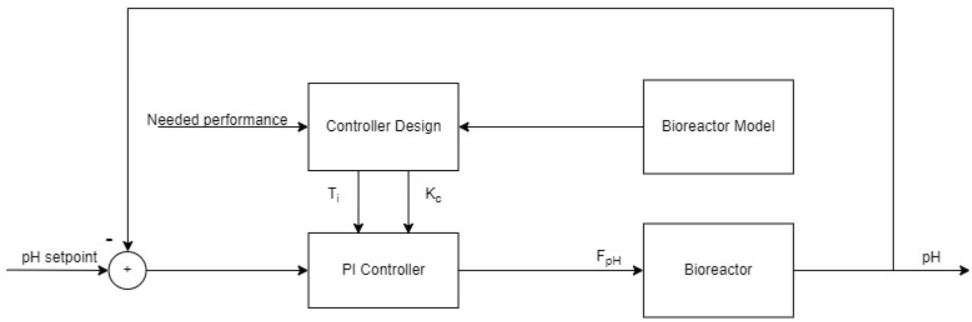


Figure 1.1. Controller design for pH control procedure

The dynamic model of the bioreactor can be determined from experimental data. It could be said that the development and fine-tuning of the controller is performed from the information gathered from the system. The tuning and development of the above mentioned system close to real time can be considered as a realization of an adaptive control system. Online data from the process will then be used to adapt and fine-tune the controller parameters online. The equivalent adaptive control system is illustrated in Figure 1.2.

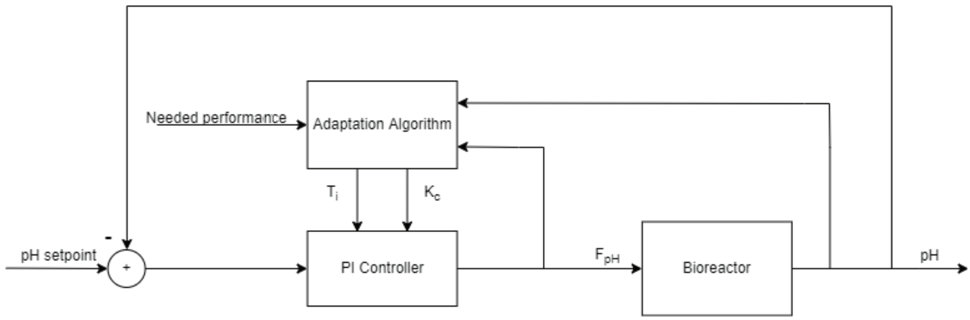


Figure 1.2. Adaptive controller design for pH control procedure

Various adaptation techniques can be described based on the way how information is processed and used for controller parameter adaptation. Figure 1.2 depicts that an adaptive control system is non-linear given that the parameters of the controller will rely upon measurements of the system variables through the adaptation loop. An example of the case would be a conventional feedback control loop designed to track the selected setpoint pattern. When a disturbance occurs and the controlled variable drifts from its setpoint, the drift of the controlled variable in the direction of its nominal value will be described by the desirable damping if the plant parameters have their known nominal values. If they alter due the disturbances, the damping of the system response may change. After an adaptation loop has been implemented, the system response damping will be retained even though the parameter values change over time.

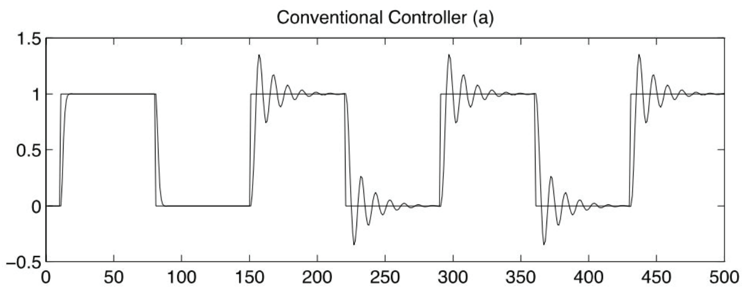


Figure 1.3. Conventional controller response to change in plant parameters [18]

Figure 1.4 depicts the functioning of an adaptive controller. Figure 1.3 depicts how the plant model parameters change at t = 150. In this case, the used controller features constant parameters. It can be seen that oscillations occur as a result of change in this parameter.

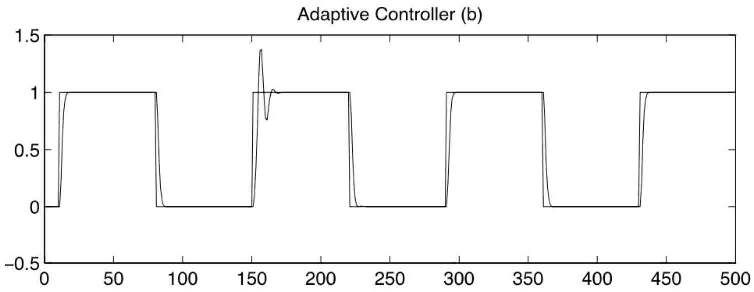


Figure 1.4. Adaptive controller response to change in plant parameters [18]

Figure 1.4 illustrates the operation of an adaptive controller in the above discussed example process. The disturbance is dealt with, and the nominal performance is restored.

1.3. Direct adaptive control

Definition of the control loop performance is one of the most important aspects. In many instances, the expected performance of the control system can be described in terms of the characteristics of a dynamic system. This system is a realization of the desired behavior of the closed-loop system. For example, a tracking objective specified in terms of rise time, and overshoot for a step change command can be alternatively expressed as the input-output behavior of a transfer function (for example a second-order with a certain resonance frequency and a certain damping). A regulation objective in a deterministic environment can be specified in terms of the evolution of the output starting from an initial disturbed value by specifying the desired location of the closed-loop poles. In these cases, the controller is designed such that, for a given plant model, the closed-loop system has the characteristics of the desired dynamic system.

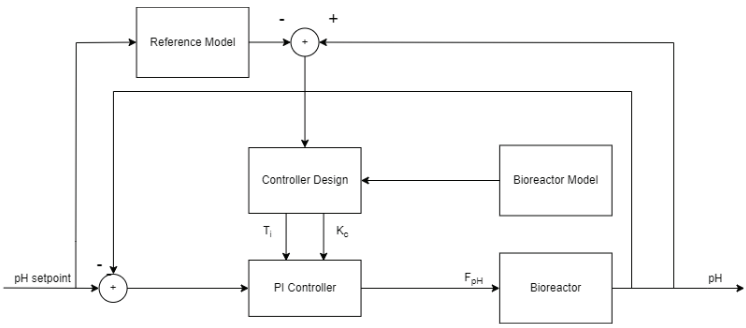


Figure 1.5. Controller design based on a reference model

This control method is based on the assumption that the plant and reference model output difference is a measure of the real and expected performance difference. This data (together with other data) is used by the adaptation algorithm to directly tune the parameters of the controller close to real time in order to force asymptotically the plant model error to zero.

Despite its charm, the usage of direct adaptive control algorithms is limited by the hypotheses related to the underlying linear design in the case of known parameters. While the performance can in most cases be described in relation to the reference model, the circumstances of the creation of a viable controller enabling the closed loop to match the reference model are limited. One of the basic restrictions is that one has to make an assumption that the plant model has stable zeros in all the situations, which, in the discrete-time case, is quite exclusive. The problem becomes even more difficult in the multi-input multi-output case. While different solutions have been proposed to overcome some of the limitations of this approach [19, 20], direct adaptive control cannot always be used.

1.4. Indirect adaptive control

Indirect adaptive control is presented in Figure 1.6.

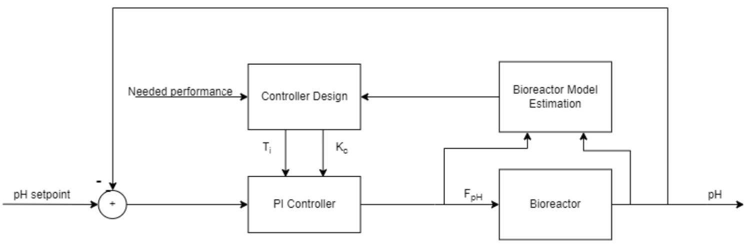


Figure 1.6. Indirect adaptive control

The basic idea is that a suitable controller can be designed online if a model of the plant is estimated online from the available input-output measurements. The scheme is termed indirect because the adaptation of the controller parameters is done in two stages:

1. Online estimation of the plant parameters.

2. Online computation of the controller parameters based on the current estimated plant model.

The indirect adaptive control scheme offers a large variety of combinations of control laws and parameter estimation techniques. To better understand how these indirect adaptive control schemes work, it is useful to consider in more detail the online estimation of the plant model [21, 22, 23].

1.5. Overview of existing techniques for adaptive control of biotechnological processes

Intense global competition, business strategies that are mainly based on profit, promptly developing social and economic conditions, high interest in better-quality control, increased safety concerns, and strict environmental norms are encouraging many process industries to automate their operations using precise, robust, trustworthy, effective, optimal, adaptive and intelligent modern control systems [12, 13, 14].

Adaptive control systems of various complexity have been developed for the automatic control of the cultivation process parameters under time-varying operation conditions. The system, based on process tendency models and online measurements of process variables [24, 25], provides high-quality control under extreme operating conditions (oxygen uptake rate rapidly changing within a wide range, feeding and aeration rate disturbances). However, the development of a model-based control algorithm is a time-consuming task, and, in addition, online measurements of the process variables require advanced instrumentation of the controlled process. Expert knowledge-driven adaptive fuzzy systems are effective; however, they require deep process knowledge [11, 26]. Fuzzy knowledge based control (FKBC) was developed by Zadeh and is based on the fuzzy set theory. It deals with a vague and unprecise class of objects, named as linguistic variables, and is described by membership functions with membership degrees that can vary between 0 and 1, fuzzy conditional statements, and the Fuzzy inference system (FIS) [27, 28]. Mamdani type and Sugeno type fuzzy logic controllers are two most known and most commonly used FKBC schemes [29, 30, 31, 32, 33]. FKBC has been extensively used to create PI, PD, and PID schemes for pH control [34, 35, 36, 37, 38, 39]. The academic community has proposed many FKBC models in association with neural networks to control pH in neutralization processes [40, 41, 42, 43]. Additionally, many modifications of gain-scheduled and self-tuned FKBC techniques are also described in academic literature [44, 45, 46, 47, 48, 49, 50]. Finally, some research works on the design of the adaptive controller for nonlinear systems using the backstepping technology have also been reported in academic literature [51, 52, 53, 54]. The adaptive FKBC developed and presented by Singh et al. is based on the Mamdani fuzzy interference system, and it uses the normalized error and the change in error as the input variables and normalized change in output as the output variable. These above mentioned variables can attain seven linguistic values and are described with 49 fuzzy rules. The developed system outperformed the other methods, however, the vast amount of membership functions and rules requires deep process knowledge and takes a lot of time to develop and fine-tune.

Model–reference adaptive control (MRAC) uses a reference model of the process that defines how the process output should respond to a command signal [55]. The purpose of the MRAC algorithm is that the system outputs of the entire plant are equal to the model reference outputs. Palancar et al. [55] proposed a MRAC model for pH control where the parameters of the model were adapted based on the gradient algorithm [56, 57]. The developed system tended to perform well with a short/moderate delay time (<6s). Even though MRAC is a good replacement for PID, it requires tuning for each individual process. However, this procedure depends on the process lag/delay as well as some elements. In the case of unknown processes, the controller needs to be tuned experimentally, and that would be a drawback from the commercial or business perspective [11].

Table 1.1. Summary of control algorithms presented by the academic community

Author	Model type	Controlled variable	Reference
Levišauskas et al.	Process tendency models	DOC	[24, 25]
Palancer et al. Kang et al. Alsabbah et al. Karasakal et al. Heredia et al. Kannngot et al.	Fuzzy logics	pH	[34, 35, 36, 37, 38, 39]
Jang et al. Eikens et al. Chen et al. Alkamil et al.	Neural Networks and Fuzzy	pH	[40, 41, 42, 43]
Adroer et al. Fuente et al. Babuska et al. Venkateswarlu et al. Salehi et al. Nsengiyumva et al.	Gain Scheduling	pH	[44, 45, 46, 47, 48, 49, 50]
Yu et al. Zhou et al. Xiang et al. Nejati et al.	Backstepping technology	pH	[51, 52, 53, 54]
Palancer et al.	Model Reference Adaptive control	pH	[55]
Galvanauskas et al. Mohan et al. Steinwandter et al. Sagmeister et al.	Gain Scheduling Model Reference Adaptive control Model Predictive control Neural Networks	Specific growth rate	[58, 59, 60, 61]

Several gain-scheduling approach-based control systems have been developed for adaptive control of batch bioreactors. Gain scheduling can be described as a modern PID control method that is highly suitable for nonlinear process control where the process dynamics are changing together with the operating conditions [62, 63]. A fitting example would be biotechnical production processes where the dynamics change significantly during different stages of the cultivation process [64, 65]. Gain-scheduling control is able to cope with rapid process dynamic changes if the values or estimates of the quantities describing the related changes in dynamics can be monitored with an adequately small lag. In the control systems presented by Cardelo and

Kuprijanov, the oxygen uptake rate (OUR) [62, 66] and the carbon dioxide evolution rate (CER) [67] are used as gain-scheduling variables. In the control systems, the OUR and CER are estimated from the online analysis of the exhaust gas. A requirement for the practical realization of the above systems is that the bioreactor system is equipped with the exhaust gas analyzer.

A summary of the various control algorithms presented by the academic community can be found in Table 1.1. The analysis shows that pH, DOC and SGR are the most common controlled parameters, and they shall be therefore covered in this disertation.

1.5.1. Specific growth rate control

One of the most important control engineering tasks is the development of straightforward and robust methods that could be used to monitor and control the specific growth rate (SGR) in industrial bioreactors. This is often needed to successfully implement a Process Analytical Technology (PAT) framework in bioengineering [68]. Nevertheless, currently, mostly relatively basic control systems are being used in most industrial-scale bioreactors, even though advanced control strategies are being extensively discussed in the academic community [15].

The biomass SGR, which can be described as the ratio between the biomass absolute growth rate and the biomass amount accumulated in the culture broth, can be considered as one of the most important variables in biotechnological processes. Not only does SGR influence the physiological state of a microbial culture, it also defines the production of the desired products, their quantity, and quality [69, 70, 71], and also the rate of product synthesis. There are two methods to obtain the SGR value. The initial approach involves determining the rate of change in dry biomass samples, which can be a time-consuming process taking several hours or even days. Consequently, this technique is unsuitable for providing feedback to a control system. Alternatively, the SGR value can be estimated by using soft-sensors [72, 73] which utilize other measurable online parameters, such as OUR. Such effects as substrate inhibition or overflow are fairly common in fed-batch bioreactors. However, these effects can be dealt with if controlled properly. A well set-up control system is able to yield high product concentrations as well as high cell densities. This can be reached by maintaining the substrate concentration at certain levels leading to a controlled biomass growth rate [12] that can be achieved by changing the substrate feeding rate.

In many cases, the specific growth rate is controlled by using PID controllers which usually operate in basic control systems. Control quality gravely relies on the development and tuning of the controller and its ability to deal with process variability and disturbances [12, 14, 13]. The common PID controllers which use constant tuning parameters are unable to achieve the required control accuracy of the process since the dynamics significantly vary during the operation. The academic community has proposed various PID controller parameter tuning approaches that take into account time-varying operating conditions: rule-based fuzzy systems [26], first-principle models [58], gain scheduling methods [58], as well as many other techniques [12, 13, 59, 60, 61, 74, 75, 76]. For exponentially evolving processes, a technique based on feedforward-feedback control has been proposed [77]. Babuska et al. proposed a supervised model based self-tuning control system that used fuzzy logics to re-tune a PID controller or to re-model and re-identify the system if degradation is noticed in the control performance. A summary of different methods used to control SGR is presented in Table 1.2.

Table 1.2. Summary of SGR control algorithms presented by the academic community

Author	Model type	Year	Ref.
Babuška et al.	rule-based fuzzy systems	2003	[26]
Bouyahia et al.	AGSMC	2020	[78]
Galvanauskas et al.	first-principle models gain scheduling MFA	2019	[58]
Galvanauskas et al.	fuzzy system with feedforward compensator	2022	[79]
Brignoli et al.	Feedforward-feedback	2020	[77]
Kottelat et al.	Calorimetry-based control	2021	[76]
Bala et al.	Fuzzy logic controller	2022	[80]

Even though various complex control models have been presented in the academic community, it is still common to use simple control systems in industrial control [81]. Even though bioprocess productivity and quality could be improved, it is still common to underestimate the importance of control systems. High costs of implementation and maintenance are still one of the main reasons why plant managers decide to avoid the implementation of these advanced systems.

1.5.2. Dissolved oxygen concentration control

Today, microorganisms play a vast role in the production of commercial products. Living cells are grown to large numbers and made to produce the desired substance, most commonly, a protein. The cells are cultivated in a bioreactor where numerous control loops secure that important process variables, such as the temperature or pH, stay within the specified operating ranges. In aerobic processes, it is also crucial to provide the culture with oxygen. The most common way to provide enough oxygen is to keep a constant DOC value. It is often acceptable to hold the DOC above a predefined level, and the performance demands on the DOC control are then moderate.

Some applications have restrictions for DOC values due to the fact that high DOC levels could be toxic for some microorganisms.

There are various ways to control dissolved oxygen in a stirred bioreactor:

- flow rate manipulation;
- oxygen concentration in the incoming air manipulation;
- reactor pressure manipulation;
- stirrer speed manipulation.

The stirrer speed is the most common manipulated variable for dissolved oxygen concentration control. Batch and fed-batch mode cultivation are known for their dynamic parameter variation. This variation may lead to tuning problems when fixed parameter controllers are being used and high performance is expected. Therefore, various adaptive DOC control techniques have been proposed by the academic community (Table 1.3).

Table 1.3. Summary of DOC control algorithms presented by the academic community

Author	Model type	Year	Reference
A. Mészáros et al.	ANN	2004	[82]
Du, Xianjun et al.	Radial basis function neural network	2018	[83]
Levišauskas et al.	First-principle models Statistical analysis	2019	[84]
Villadsen et al.	Feedforward-feedback	2020	[85]
Bo et al.	Echo state nettworks	2018	[86]
Akisue et al.	Fuzzy logic controller	2018	[87]

An approach of development for the control systems of dissolved oxygen concentration (DOC) and pH based on artificial neural network (ANN) models is presented in [82, 83]. A. Mészáros et al. present ANNs that are trained offline to predict the non-linear dynamics of controlled processes, and inverse ANNs are used in the control systems as feedback controllers [82]. Du, Xianjun, et al. developed a radial basis function neural network based adaptive PID controller for DOC control [83]. Such development of ANN model-based control systems requires a sufficient amount of informative process data and time expenses for training the ANNs. Bo et al. proposed an echo state network based control method [86]. The system consisted of tree modules and required an online learning algorithm on the basis of recursive least squares. This type of models are complex and require a lot of time to fine-tune and develop. For these reasons, the application of complex control systems is not attractive in the industrial bioprocess-control engineering practice.

DOC control systems have also been developed [88, 84], in which the PID (PI) controller adaptation does not require additional measurements of process variables. The controller adaptation is based on the online statistical analysis of the controller input and output data. Computer simulations of the control systems performance show the working capacity of the adaptation algorithms. However, the optimal values of the algorithm tuning parameters are determined by the 'trial and error' approach which is time-consuming. Various other PID controller-tuning approaches were presented in [89, 74, 75]. A feedforward–feedback controller was proposed in [85] in which processes that evolve exponentially were controlled.

1.5.3. pH control

Adjusting the pH level helps to ensure product quality and reduces the amount of equipment in the controlled object corrosion. Sufficiently effective pH control saves the reagents used for controlling the pH level. pH level control is used in various fields:

1. In biotechnology, pH control is applied to the cultivation of microorganisms and metabolism [90].

2. In the chemical industry, for salt crystallization processes [91].

3. In surface treatment processes, for the efficient removal of paints and varnishes, the hardness of nickel coatings, and for the selection of the brightness of nickel coatings [92].

4. In fermentation processes, for the regulation of the fermentation time and the growth of organisms in fermentation processes [93].

5. In pharmaceuticals, for studies on body reactions, stability and efficacy of formulations [94].

6. For water softening, purification processes and other related applications [95, 96].

High quality control of pH is difficult because of many reasons: very strong non-linearity of biochemical processes, titration curves and pH measurement itself, high sensitivity of the microorganisms even to small temporary deviations of the pH level in the cultivation media, and drift of the pH sensors [97, 98].

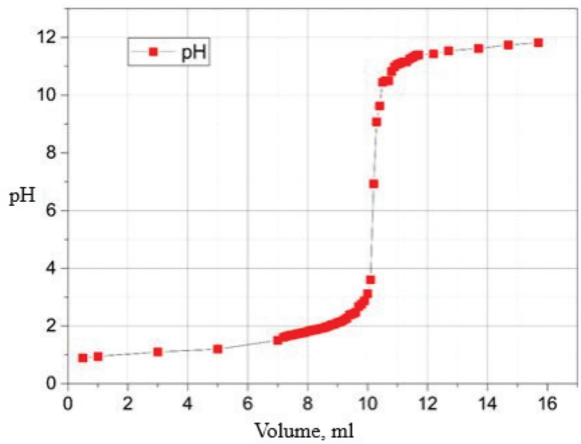


Figure 1.7. Example of a titration curve

From the titration curve, it can be seen that the addition of a base solution to the acid solution, and vice versa, changes the pH non-linearly, and the rate of this variation increases significantly in the range from pH = 3 to pH = 11 depending on the strength of the solution and the reagent added. Therefore, even a slight deviation of the reagent can prompt drastic pH deviations. For comparison, when using a strong acid and alkali reagents system, the order of magnitude of the measurements of the titration process at pH = 6...7 is 6 orders of the magnitude higher than the measurements when pH is between 0 and 1.

The academic community has proposed various PID controller parameter tuning approaches for high-quality pH control (Table 1.4).

Table 1.4. Summary of pH control algorithms presented by the academic community

Author	Model type	Year	Reference
Henson et al.	input/output linearization	1994	[99]
Yloestalo et al.	self-organizing fuzzy controller	2001	[100]
Yan et al.	nonlinear model predictive control	2022	[101]
Kim et al.	model predictive control	2023	[102]
Nsengiyumva et al.	self-adaptive fuzzy controller	2018	[50]
Galvanauskas et al.	gain scheduling	2009	[103]

Henson et al. [99] proposed an adaptive nonlinear control strategy for pH control. The non-linear controller development is based upon an adjusted input/output linearization method which considers the implicit output formula in the reaction invariant model. The adaptive control algorithm was implemented by expanding the standard controller with indirect parameter approximation based on unmeasured buffering changes. Yloestalo et al. [100] compared several adaptive pH control algorithms

– the standard PID controller, the controller based on the algorithm developed by Kurz [104], a self-organizing fuzzy controller, and a multimodel controller that is based on a self-organizing map. All the presently mentioned controllers outperformed the standard PID controller. Kim et al. proposes a model predictive control based system for parallel mini-bioreactors [102]. Nevertheless, most of the discussed control algorithms suffer from some drawbacks, such as a complex controller design, huge time investments for development, expensive hardware, or excessively many tuning parameters. On the other hand, well-functioning pH control systems can be used to monitor biological reaction rates [105]. Therefore, it is of primary importance to elaborate simple, robust, and easy to implement methods for precise pH control.

1.6. Conclusions of the chapter

1. Biotechnological process control is one of the most complex control types due to its characteristics: nonlinear relationships between the process variables, time varying dynamic properties, lack of inexpensive and reliable sensors, and knowledge of complex process.

2. Standard PI(D) controllers are used in the industrial biotechnological process control even though many advanced control solutions have been proposed by the academic community. The proposed advanced solutions tend to be complex, therefore, they are not attractive for industrial applications.

3. Basic PI(D) controllers are not able to cope with the changing conditions of the controlled process. Adaptation of the controller parameters is needed to keep up with the changing conditions and to maintain or increase the performance.

4. The adaptive controllers need to rely on simple measurements and be easily implementable in the industry. They should be user-friendly and relatively simple, compatible to standard control and measurement equipment, easy to tune and to develop. Their performance should be compared with the standard PI(D) controller performance since most of industrial biotechnological processes are still controlled with the conventional PI(D) controllers with fixed parameters. Based on these observations, gain-scheduling, Fuzzy logic, statistical analysis and polyno-mial-based PI controller parameter adaptation algorithms shall be developed.

5. Specific growth rate, pH, dissolved oxygen concentration and temperature are the most commonly controlled variables in the biotechnological process control.

6. The Effective control of these process variables is needed to maximize the performance of biotechnological processes.

2. PROPOSED METHODS FOR ADAPTIVE CONTROL OF BIOTECHNOLOGICAL PROCESSES

2.1. Adaptive control algorithm selection

In this doctoral dissertation, several adaptive control algorithms shall be presented. To help the user in the selection between the different developed adaptive control algorithms, activity diagrams in UML were created for each of the investigated control variables.

2.1.1. SGR control

When selecting an adaptive control algorithm for SGR control, the process and mathematical knowledge need to be taken in to account.

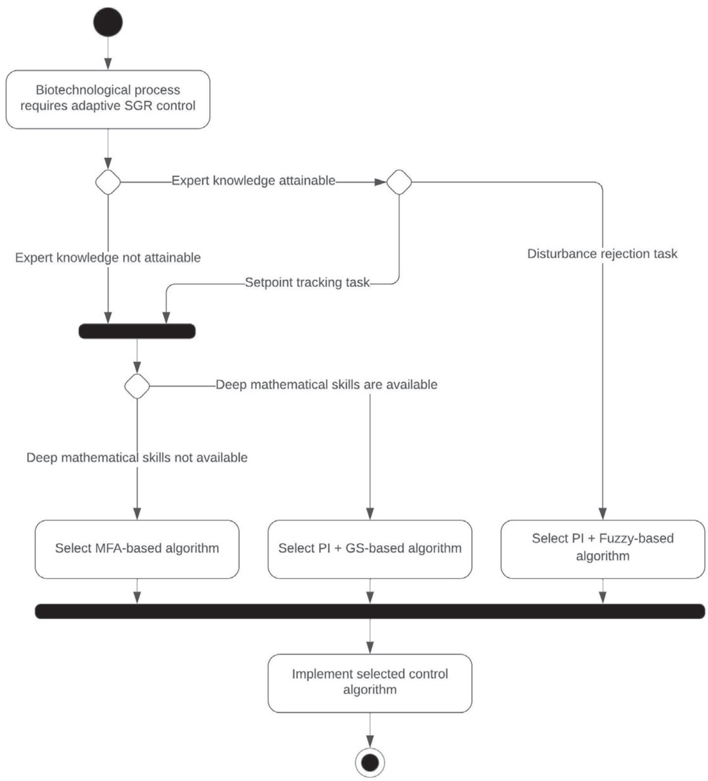


Figure 2.1. SGR adaptive control algorithm type selection UML activity diagram

Fuzzy knowledge-based PI controller parameter adaptation (Section 2.2) is more suitable for disturbance rejection tasks, and it requires process knowledge. Meanwhile, the gain scheduling or MFA-based control techniques [58] require deep mathematical skills or large amounts of experimental data and are more suited for the setpoint-tracking task.

2.1.2. DOC control

When selecting an adaptive control algorithm for DOC control, mathematical skills, process knowledge, and auxiliary variable measurement availability need to be taken into account.

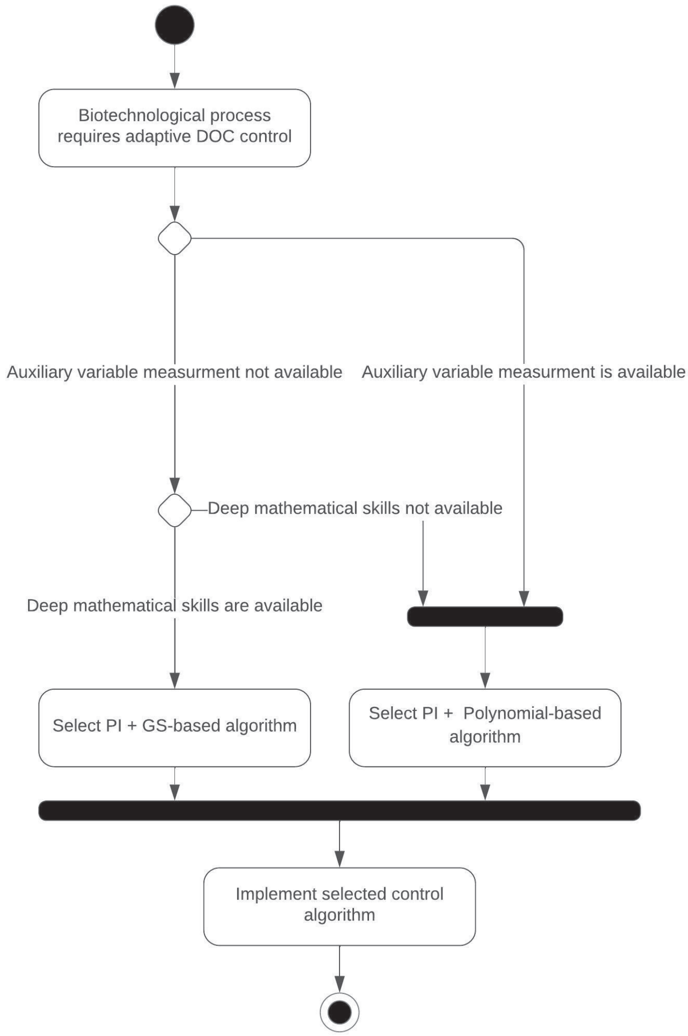


Figure 2.2. DOC adaptive control algorithm type selection UML activity diagram

The gain scheduling-based adaptive control algorithm (see Section 2.3) does not require additional measurement equipment, and it relies only on the controller input/output variables, thereby making it attractive for the typical DOC control processes. In such cases when deep mathematical and process knowledge is not available, the polynomial-based control algorithm (see Section 2.6) can be used. However, this algorithm requires OUR values for the adaptation of the controller parameters; therefore, addition soft-sensors are needed to implement this algorithm.

2.1.3. pH control

When selecting an adaptive control algorithm for pH control, the auxiliary variable measurement availability and mathematical knowledge need to be taken into account.

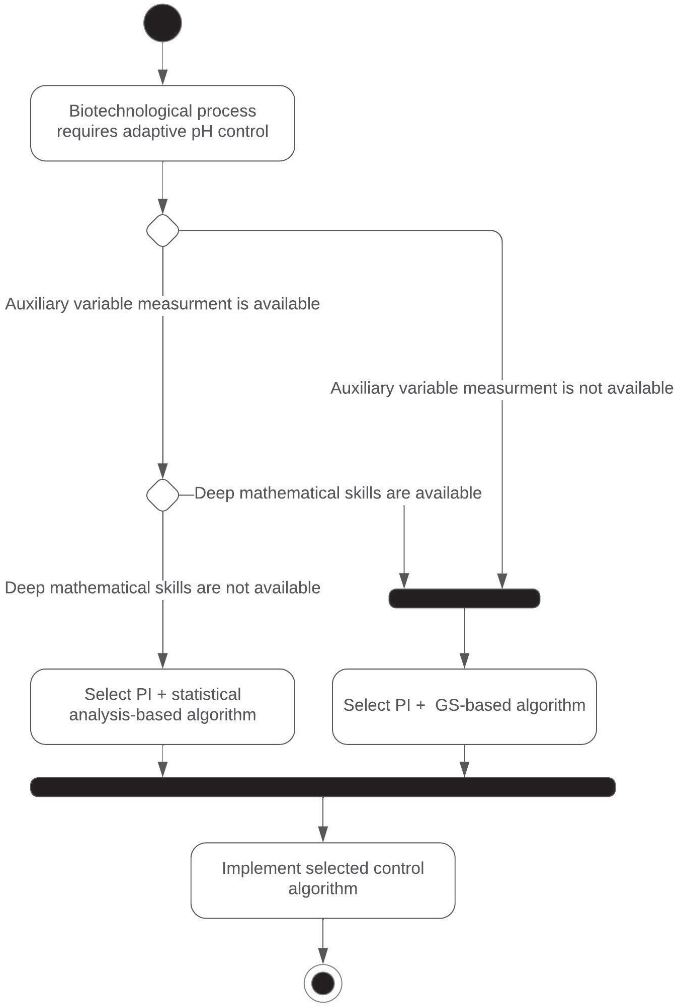


Figure 2.3. pH adaptive control algorithm type selection UML activity diagram

The gain scheduling-based adaptive control algorithm (see Section 2.4) does not require any auxiliary variable measurement equipment, and it relies only on the controller input/output variables. The statistical analysis-based control algorithm (see Section 2.5) requires a feed forward compensator which uses OUR values. Therefore, this method relies on external parameter measurement, but does not require deep mathematical skills and process knowledge to develop the control algorithm.

2.2. Fuzzy logic-based adaptive control of specific growth rate

2.2.1. Mathematical model of the biotechnological process

In this study, the biotechnological process was simulated by using the mathematical model of the *Escherichia coli* BL21 strain. This strain also harbored a pBR322 plasmid derivative and was cultivated in a recombinant fed-batch process, mainly because this recombinant protein is used in the typical bioreactor-scale processes. The cultivation of the selected recombinant protein can be described as a two-phase process. The target of the first phase is to accumulate the bioreactor with biomass. The specific growth rate is usually relatively high for this phase. During phase two, the recombinant protein is produced. During the described two phases, different optimal temperatures were defined and maintained for each phase. In the simulated process, the broth temperature was maintained at 37 °C during the first phase, thus maximizing the biomass growth to the optimal level. At the start of the production process, the temperature was reduced to 32 °C. The substrate feeding rate profiles and the induction time also exert significant influence on the process performance. Therefore, they are also subject to model-based optimization. The induction time (8h) for the investigated process of a given total duration was determined in [106] by using model-based optimization techniques. During the model-based optimization of the process [106], the process performance (productivity) index equal to the total target protein amount at the end of the process was used. The online measurement techniques applied for biomass and target protein analysis in the investigated process are described in detail in [106]. For the online measurements of the biomass concentrations, alternative techniques based on turbidity (optical density), permittivity measurements or off-gas analysis can be used. For protein measurement, a promising alternative to the applied techniques are the model-based approaches (application of soft-sensors, generic estimators, e.g. [107]) that would enable the online monitoring of the key process variables.

A mathematical model presented in [106] was used to simulate the described biotechnological process in *Matlab*. Since the model has already been identified, no statistical analysis of the model parameters is carried out in this work. According to other studies, preoptimized feeding rate profiles are used to control the biomass growth and protein production [106]. This type of an open-loop system can be controlled effectively only when no considerable process condition deviations or equipment disturbances/malfunctions are present. Failure to do so may lead to deviations in the specific growth rate, thus reducing the process productivity. Therefore, SGR was selected as the main controlled variable that would be maintained by controlling the substrate feeding rate. An adaptive closed-loop control algorithm was used for keeping the optimal SGRs trajectory on track. The discussed process can be described by using the below provided differential equations.

$$\frac{dx}{dt} = \mu(s,T)x - u\frac{x}{w} \tag{2.1}$$

$$\frac{ds}{dt} = -q_s(s,T)x + u\frac{S_0 - s}{w} \tag{2.2}$$

$$\frac{dp_x}{dt} = q_{px}(\mu, p_x) \tag{2.3}$$

$$\frac{dw}{dt} = u + F_{smp} \tag{2.4}$$

where x (g/kg) describes the biomass concentration. μ (1/h) is the biomass specific growth rate that is a function of the glucose concentrations s (g/kg) and the culture broth temperature T ($°C$). Here, w (kg) is the culture broth weight, q_s (g/(gh)) is the glucose specific consumption rate. The glucose concentration in the feeding solution is denoted as S_0 (g/kg), while p_x (U/(g biomass)) is the specific protein activity. q_{px} (U/(gh)) is the specific protein accumulation rate; u (kg/h) and F_{smp} (kg/h) are the substrate feeding and sampling rates, respectively. SGR is modeled by using the Haldane-type model [58]:

$$\mu(s,T) = \mu_{max}\frac{s}{K_s + s}\frac{K_i}{K_i + s}\exp(\alpha(T - T_{ref})) \tag{2.5}$$

where μ_{max} (1/h) is the maximal specific growth rate parameter. K_i and K_s (g/kg) are the inhibition and Monod constants. The extent to which the temperature influences the growth rate is considered with the parameter α ($1/°C$). The optimal growth phase temperature is denoted as T_{ref} ($°C$). The optimal temperatures which yield the highest growth and protein synthesis rates were determined in the study by Volk et al. [108]. The specific consumption rate of the substrate q_s is given by the following expression:

$$q_s(s,T) = \frac{1}{Y_{xs}}\mu(s,T) + m \tag{2.6}$$

where m (g/(gh)) is a maintenance term and Y_{xs} (g/g) is the conversion yield coefficient. This consumption rate is proportional to the cell growth and rate of substrate consumption. Negative concentration of the substrate is avoided by implementing saturation (s>=0) in the simulation code. The vital functions of the cell are also considered by implementing a maintenance term.

According to the research on various recombinant proteins as target products, the SGR and the temperature are stated as the essential parameters which influence the protein production rate [58]. However, it could be that both parameters, depending on the particular target product, may differ. Inclusion body formation and soluble protein formation could be taken as an example, since the first requires a relatively high value of the SGR compared to the second process [108, 109, 110, 111, 112, 113]. In the simulation, the mathematical model of the target accumulation rate of the product q_{px} considers the actual protein activity and the influence of the SGR:

$$q_{px}(\mu, p_x) = \frac{1}{T_{px}}(p_{max}(\mu) - p_x) \tag{2.7}$$

$$p_{max}(\mu) = \frac{\mu K_m}{K_\mu + \mu + \mu^2/K_{i\mu}} \tag{2.8}$$

where T_{px} (h) is the protein accumulation time constant. The maximal specific protein activity is defined as $p_{max}(\mu)$(U/(g biomass)). This parameter also depends on the SGR: K_m (U/(g biomass)), K_μ (1/h) and $K_{i\mu}$ (1/h) are the Monod and inhibition constants, respectively. The oxygen uptake rate OUR (g/h) was described by using the Luedeking–Piret-type model [113, 114]:

$$OUR = (Y_{ox}\mu + m_{ox})xw \tag{2.9}$$

where Y_{ox} (g/g) is a coefficient describing the conversion yield, and m_{ox} (g/(gh)) is a maintenance term. Equations (2.1)-(2.9) were used for modelling the behavior of the described fed-batch process and to analyze the proposed adaptive closed-loop control algorithm's control performance. In this simulation experiment, it is presumed that the bioreactor is ideally mixed and that neither actuators nor measurement devices cause significant time delays which may influence the control quality. The model parameter values identified in [106] were used in this simulation and can be found in Table 2.1.

Table 2.1. Model parameters used in the simulation experiment [106]

Model parameters			
K_i = 93.8 g/kg	$K_{i\mu}$ = 0.0174 1/h	K_m = 751 U/g	T_{ref} = 37 °C
K_μ = 0.61 1/h	m = 0.0242 g/gh	m_{ox} = 0.05 g/gh	S_0 = 151 g/kg
T_{px} = 1.5 h	K_s = 0.0033 g/kg	Y_{ox} = 0.7 g/g	Y_{xs} = 0.46 g/g
	α = 0.0495 1/°C	μ_{max} = 0.737 1/h	

If the time constant of a process falls in the range of minutes to hours, and the duration of cultivation is lengthy, it implies that any changes in the process transpire at a slow pace. As a result, it is possible to consider the process as continuous provided that the controller's discretization step is below 1 second. Therefore, controller speed or delays do not influence the performance of the control algorithm.

2.2.2. Adaptive control algorithm development

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

The application of an ordinary PID controller is limited due to the non-stationary and non-linear behavior of the described biotechnological processes. Adaptation of the controller parameters may be needed because of the dynamic response variation considering the systems operation point. To fulfill the control quality and stability requirements, adaptation of the controller parameters is needed. This can be realized by using adaptive control techniques. Therefore, a fuzzy model was designed and implemented to adapt the PI controller parameters throughout the cultivation. Since the main goal of the fuzzy system is to recalculate the PI controllers tuning parameters, a classical Fuzzy system is sufficient for this task. The usage of a more complex dynamic Fuzzy system would slow down the response and increase the complexity of the system. As the SGR cannot be directly measured online, a state estimator and an auxiliary variable for the calculation of the signal were used [58]. These variables are then used for the feedback loop and fuzzy model input. In this simulation experiment, it was decided that the OUR would be a suitable additional variable due to the fact that it not only reflects the physiological state of the cultures, but it has good correlation with the SGR. Nevertheless, it is possible to accurately measure it during a bioreactor-scale cultivation process.

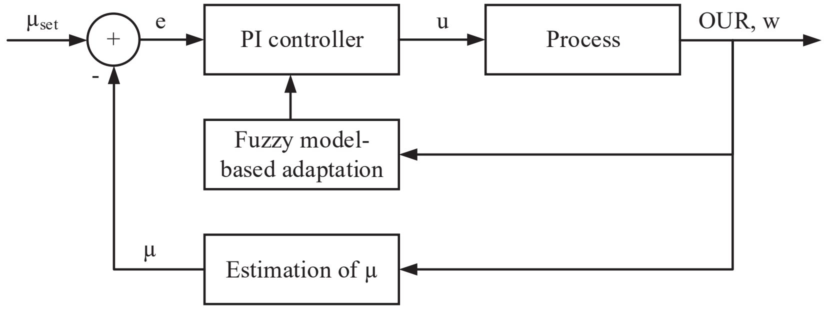


Figure 2.4. General structure of the fuzzy-based adaptive PI control system for specific growth rate control

Figure 2.4 represents the general structure of the developed fuzzy-based PI controller. The *Process* function block part of the presented scheme represents the biotechnological process that is modeled using the validated model presented in [106]. Function block *Estimation of* μ is carried out by using the soft-sensor presented in [58]. To create the *Fuzzy model-based adaptation* function blocks, the input and output variables for the fuzzy system need to be defined. Since the function of the fuzzy model is to adapt the PI controller parameters, controller gain K_c and integration time constant T_i parameters were selected as the output variables. The classical fuzzy system is selected for implementation since the dynamic fuzzy system would increase the complexity of the algorithm. Both T_i and K_c depend on the process variables as described in [58]:

$$K_c \propto k_0 w \tag{2.10}$$

$$T_i \propto \frac{k_1}{OUR/w + k_2} \tag{2.11}$$

where k_0, k_1 and k_2 are fixed parameters, the values of which are subject to optimization and were calculated and discussed in detail in [58]. The aim of the optimization was to find a set of parameters which minimizes the tracking error (e.g., the integral time absolute error (ITAE)) of the designed control system when using Equations (2.10) and (2.11) for controller parameter adaptation. Since the PI controller parameters mainly depend on OUR and w, both process variables need to be used in the fuzzy system. To decrease the complexity of the fuzzy system, the ratio of these two process variables was used as the input for the fuzzy system.

According to the heuristic knowledge available, the following rules were created for the fuzzy system:

IF OUR/w is poor, THEN T_i is big

IF OUR/w is excellent, THEN T_i is small

IF OUR/w is poor, THEN K_c is small

IF OUR/w is excellent, THEN K_c is big

Both for the input and the output of the fuzzy model, two Gaussian membership functions were selected. To avoid the extensive overlapping of the membership functions, the number of the membership functions used per parameter was decreased to two as seen in Figure 2.5.

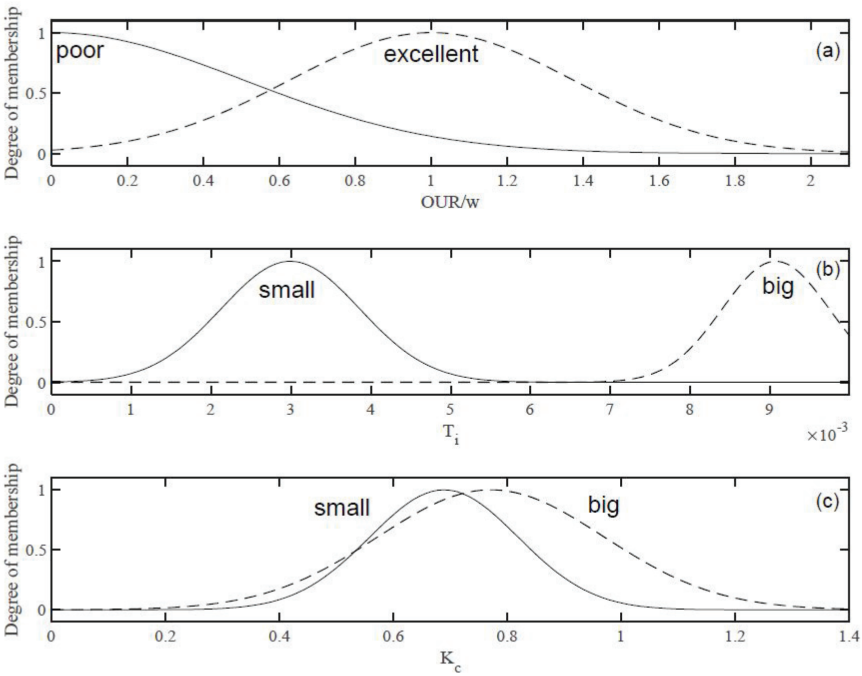


Figure 2.5. Fuzzy model membership functions: model input membership functions (a), model output T_i membership functions (b), model output K_c membership functions (c)

Whether a fuzzy application is implemented successfully depends on a number of factors. One of these are the membership functions of the fuzzy model. However, these are usually decided upon subjectively. One way to improve the performance of the fuzzy model is the use of the genetic algorithm (GA). Various studies have shown that GAs and other heuristic algorithms are able to improve the system's performance by optimizing the membership functions [115, 116, 117]. Due to its simplicity and straightforwardness, a genetic algorithm was developed to find the optimal membership function parameters. GAs adapt a direct analogy of the natural evolution to perform global optimization in order to solve highly complex problems. They assume that the possible problem solution is individual and can be described by a set of parameters. These parameters are coded as genes of a chromosome and can be structured by a string of concatenated values. Variable representation is defined by the encoding scheme that can be represented by various forms, such as binary or real numbers, depending on the data in use. The search space of the data is usually defined by the problem. In this simulation experiment, the fuzzy model membership function parameters were coded in the chromosomes. At the beginning, an initial chromosome is randomly generated. Then, the fitness values of all chromosomes are evaluated by calculating the objective function in the decoded form. Since the process performance depends on the tuning of the controller, the integral time absolute error (ITAE) value of the SGR setpoint tracking was selected as the fitness function since this criterion was also used for the system's performance evaluation. After getting determined by the fitness of each individual, the best chromosomes are grouped, based on the lowest ITAE value, and the group with the lowest value is then selected in the selection process. Such genetic operators as crossover and mutation are applied to this selected population in order to improve the next generation solution. The process is repeated until the population converges to the global minimum, or another termination criterion has been reached. In the reproduction phase, the fitness value of each chromosome is evaluated, and it is used in the selection process to provide bias towards fitness individuals. Then, a crossover algorithm is initiated once the selection process has been completed. The background operator in genetic algorithms is mutation. The parameters of the GA directly depend on the number of variables. In the recommendations provided by the authors of the GA, the number of generations should be equal to $Numberofvariables * 20 + 10$, and the population number is calculated as $Numberofvariables * 50$ by default. After the manual tuning of the membership function parameters based on the heuristic knowledge of the process, 10 membership function parameters were selected for further optimization with the GA, thus simulating 210 generations with 500 populations. The initial generation was created randomly. The large number of generations did not bring significant changes, hence it was decided to use 50 generations for testing. The mutation and crossover parameters were left at their default values. Parameter identification was performed 20 times to avoid local minimums and lucky guesses. In Table 2.2, the parameters used for the GA are presented.

Table 2.2. Genetic algorithm parameters

No. of Generations	Individuals in One Generation	Mutation Probability	Crossover Probability
50	500	0.1	0.9

This GA was used to search for the optimal parameters of the fuzzy controller membership functions. Each individual represents a simulation of the biotechnological process where the PI controller parameters are adapted by using the generated fuzzy model. The structure of the controller is constant and does not change during the simulation of the process. The GA searches for the optimal fuzzy model structure by defining the membership function parameters. In the applied genetic algorithm, the only termination criterion was the predefined number of generations. This allowed avoiding early convergence and local optima. After 50 iterations, the GA was stopped as shown in Figure 2.6.

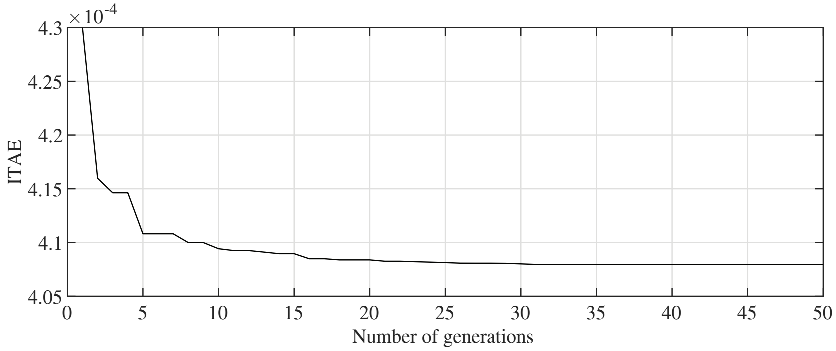


Figure 2.6. ITAE criterion change during each generation of the genetic algorithm

The developed PI controller with fuzzy-based adaptation was implemented in the model simulation and used for the performance evaluation of the system. The K_c and T_i dependencies are presented in Figure 2.7.

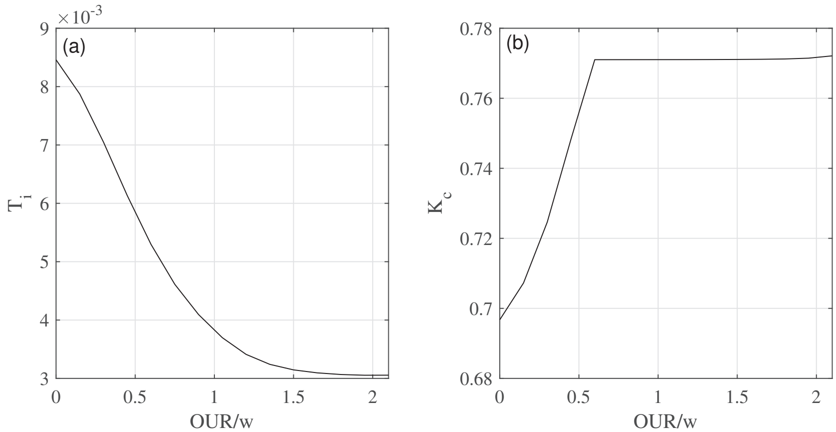


Figure 2.7. PI controller parameter dependencies generated by the fuzzy model: integration time constant T_i(a), controller gain K_c (b)

2.3. Gain-scheduled control system for dissolved oxygen control

2.3.1. Development of adaptation algorithm for DOC control

The dynamics of the dissolved oxygen concentration in culture medium can be represented by a simple tendency model based on the mass balance for DOC:

$$\frac{dc}{dt} = K_L a(c_{sat} - c) - OUR \tag{2.12}$$

where $K_L a$ is the oxygen transfer coefficient, s^{-1}:

$$K_L a = \alpha u^\beta q^\gamma \tag{2.13}$$

c is DOC, %; c_{sat} is the saturation value of DOC, %; OUR is the oxygen uptake rate, $mmols^{-1}$; u is the stirring speed (control variable), s^{-1}; q is the air supply rate, ls^{-1}; α, β and γ are parameters, -; and t is time, s. c_{sat} depends on the culture temperature, the pressure in the bioreactor and the chemical composition of the culture. During these simulation studies, it was considered that the culture temperature and pressure in the bioreactor remained stable. The pressure could be monitored by a soft sensor proposed by Survyla et al. [118].

Linearization of Equation (2.12) around the process state point at time t_k with respect to the state (c) and the control (u) variables represents the DOC dynamics equation at time t_k:

$$\frac{d\Delta c}{dt} = -[\alpha u^{\beta} q^{\gamma}]_{t=t_k} \Delta c + [\alpha \beta u^{\beta-1} q^{\gamma}(c_{sat} - c)]_{t=t_k} \Delta u \tag{2.14}$$

From Equation (2.14), the DOC dynamics can be represented by a first-order transfer function model:

$$G_{\frac{\Delta c}{\Delta u}}(s) = \frac{\Delta c(s)}{\Delta u(s)} = \frac{K_{pr}(t_k)}{T_{pr}(t_k)s + 1} \tag{2.15}$$

From this equation, the K_{pr} and T_{pr} controller parameters can be derived as:

$$K_{pr}(t_k) = [\frac{\beta(c_{sat} - c)}{u}]_{t=t_k} \tag{2.16}$$

$$T_{pr}(t_k) = [\frac{1}{\alpha u^{\beta} q^{\gamma}}]_{t=t_k} \tag{2.17}$$

$K_{pr}(t_k)$ and $T_{pr}(t_k)$ are the process controller gain and the integration time constant at time point t_k, respectively, s is the Laplace operator. The resultant dynamics of the controlled process in the DOC control system also depends on the stable dynamical parameters of the motor–stirrer system and the DOC electrode. As the time constants of the above control system elements are significantly smaller compared with the time constant $T_{pr}(t_k)$, their influence on controlled-process dynamics is taken into account by adding some time delay to the transfer function model (Equation (2.15)). Therefore, the dynamics of the DOC control process can be roughly represented by the first-order-plus-time delay (FOPTD) model:

$$G_{\frac{\Delta c}{\Delta u}}(s) = \frac{K_{pr}(t_k)}{T_{pr}(t_k)s + 1} \exp^{-\tau} \tag{2.18}$$

where τ, s is the time delay representing the influence of the dynamics of the control system elements. According to the PI controller tuning rules for static objects (Ziegler–Nichols, internal model control (IMC), etc. [113]), the controller gain K_c is proportional to the ratio $Tpr/Kpr/\tau$, and the integration constant T_i is proportional to the resultant time constant T_{pr} [119]. Taking into account the functional Relationships

(2.16) and (2.17) and assuming that the controlled value of the DOC during the cultivation process is close to the setpoint value ($c \cong c_{set}$), the character of relationships between the controller tuning parameters and the controller output and the setpoint signals can be estimated:

$$K_c(t_k) \sim T_{pr}/K_{pr}/\tau = \frac{1}{\alpha u^{\beta} q^{\gamma}} \frac{u}{\beta(c_{sat} - c)} \tag{2.19}$$

Based on Relationship (2.19), the gain scheduling algorithm for controller gain adaptation takes the following form:

$$K_c(t_k) = \frac{K_{Kc}}{(u(t_k)^{\beta-1})(c_{sat} - c_{set}(t_k))} \tag{2.20}$$

where u and c_{set} are the gain scheduling variables; K_{Kc} is the coefficient for tuning the controller to obtain desired performance of the control system (approximate values of the coefficient can be taken from the desired controller tuning rules). The power β of the stirring speed in the oxygen transfer rate estimation Equation (2.17) is typically $\beta \cong 2$ [114], and Formula (2.20) for scheduling the controller gain coefficient can be reduced to:

$$K_c(t_k) = \frac{K_{Kc}}{u(t_k)(c_{sat} - c_{set}(t_k))} \tag{2.21}$$

The character of relationships between the controller integration constant and the controller output is the following:

$$T_i \sim T_{pr} = \frac{1}{\alpha u^{\beta} q^{\gamma}} \tag{2.22}$$

Based on Relationship (2.22), the gain scheduling algorithm for controller integration time constant adaptation takes the following form:

$$T_{pr} = \frac{K_{Ti}}{(u(t_k))^2} \tag{2.23}$$

$$K_{Ti} = k_{Ti} \frac{1}{\alpha q^{\gamma}} \tag{2.24}$$

where k_{Ti} is the coefficient for tuning the controller to obtain the desired performance of the control system (the approximate value of the coefficient can be taken from the desired controller tuning rules).

2.3.2. Mathematical model of the biotechnological process

To simulate the biotechnological process, a validated mathematical model of an *E.coli* fed-batch process presented in [106] was used:

$$\frac{dx}{dt} = \mu x - \frac{F_s + F_{pH}}{V} x \tag{2.25}$$

$$\frac{ds}{dt} = -q_s x + \frac{F_s S_0}{V} - \frac{s(F_s + F_{pH})}{V} \tag{2.26}$$

$$\frac{dV}{dt} = F_s + F_{pH} - F_{smp} \tag{2.27}$$

$$\mu = \mu_{max} \frac{s}{K_s + s} \frac{K_i}{K_i + s} \frac{c_a}{c_a + k_c} \tag{2.28}$$

$$q_s = \mu / Y_{xs} - m \tag{2.29}$$

$$F_s = \frac{\mu_{set} x V}{Y_{xs}(S_0 - s)} \tag{2.30}$$

where x is the biomass concentration in the cultivation medium, gl^{-1}; μ is the biomass specific growth rate, lh^{-1}; V is the cultivation medium volume, l; S_0 is the substrate concentration in feed, gl^{-1}; F_{smp} is the sampling rate, lh^{-1}; Y_{xs} is the biomass/-substrate yield coefficient, gg^{-1}; c_a is DOC in absolute units, mmol l^{-1}; k_c is the parameter, mmol l^{-1}. The Luedeking–Piret model was used to calculate the oxygen uptake rate [113]:

$$OUR = \mu Y x V + m x V \tag{2.31}$$

The values of the model parameters are given in Table 2.3 and were selected from the ranges described in literature [25, 106, 103].

Table 2.3. DOC model parameter values and initial conditions of the state variables [25, 106, 103]

	Model Parameters	
Y = 0.8646 gg^{-1}	m= 0.018 gg^{-1}h^{-1}	Y_{xs} = 0.52 gg^{-1}
μ_{max} = 0.737 lh^{-1}	K_i = 93.8 gl^{-1}	S_0 = 450 gl^{-1}
k_c = 0.00265 mmol l^{-1}	K_s =0.02 gl^{-1}	F_{smp} = 0.025 lh^{-1}
	Initial Conditions	
$V(0)$ = 45 L	$x(0)$ = 0.25 gl^{-1}	$s(0)$ = 0.5 gl^{-1}

A set of equations is used to model and simulate the controlled process in *Matlab/Simulink*. A detailed explanation of the development and validation of these models is presented in [25]. The dynamics of the air supply and stirring systems is modeled by Equations (2.32) and (2.33). Equations (2.34) and (2.35) represent mass balances on oxygen in liquid and gaseous phases. Equations (2.36) and (2.37) are used to model the second-order dynamics of the DOC electrode:

$$\frac{dq}{dt} = \frac{1}{T_q}(q_{set} - q) \tag{2.32}$$

$$\frac{du}{dt} = \frac{1}{T_u}(u_{set} - u) \quad (2.33)$$

$$\frac{dc_a}{dt} = -OUR_v + \alpha u^\beta q^\gamma (\frac{y_{O_2}}{H} - c_a) \quad (2.34)$$

$$\frac{y_{O_2}}{dt} = \frac{q}{V}(\frac{1}{\epsilon} - 1)(0.21 - y_{O_2}) - \alpha u^\beta q^\gamma (\frac{1}{\epsilon} - 1)(\frac{y_{O_2}}{H} - c_a) v_{mol} \quad (2.35)$$

$$\frac{da_{el}}{dt} = \frac{1}{T_{el1}}(100\frac{c_a H}{0.21} - a_{el}) \quad (2.36)$$

$$\frac{dc_{el}}{dt} = \frac{1}{T_{el2}}(a_{el} - c_{el}) \quad (2.37)$$

where q_{set} is the set value of the air supply rate, lh^{-1}; u_{set} is the set value of the stirring speed (the control variable), h^{-1}; y_{O_2} is the portion of oxygen in exhaust gas, -; OUR_v is the volumetric oxygen uptake rate, mmol l^{-1} h^{-1}, a_{el} is the auxiliary variable, %; c_{el} is the signal from dissolved oxygen (DO) electrode, %; H is Henry's constant, l mmol^{-1}; V is the volume of the cultural liquid, l; v_{mol} is the volume of mmol of gas, l mmol^{-1}; T_q, T_u, T_{el1}, T_{el2} are the time constants of the air supply system, the motor-stirrer system, and the DOC electrode, respectively, s; ϵ is gas holdup in the gas-liquid dispersion, -.

The DOC model parameter values and the initial conditions of the state variables are given in Table 2.4. The parameters of the model equations are taken from the ranges reported in literature [85, 25].

Table 2.4. DOC model parameter values and initial conditions of the state variables [85, 25]

	Model Parameters	
H = 0.7906 l mmol^{-1}	ϵ = 0.15	T_{el1} = 10 s
T_{el2} = 2 s	T_q = 2 s	T_u = 1 s
α = 0.8e-7	β = 2	γ = 0.2
	v_{mol} = 0.0224 l mmol^{-1}	
	Initial Conditions	
$c_{el}(0)$ = 10%	$q(0)$ = 2 s^{-1}	$u(0)$ = 2.5 s^{-1}
$c_a(0)$ = 0.0266 mmol l^{-1}	$y_{O2}(0)$ = 0.2099	$a_{el}(0)$ = 10%

Since previously validated mathematical models which do not have any black box elements are used, no additional statistical analysis of the models in use is performed. A scheme of the DOC control system is depicted in Figure 2.8.

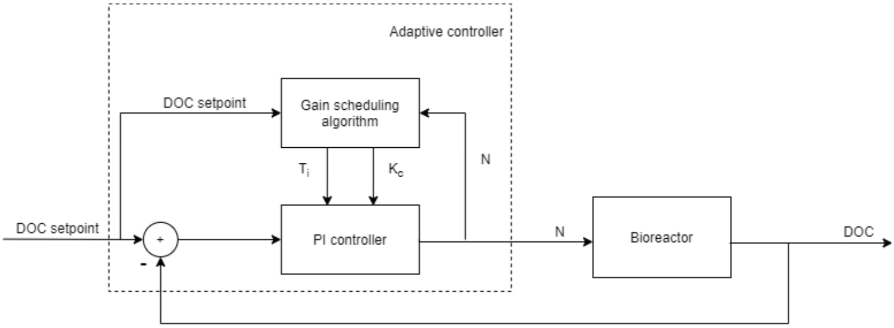


Figure 2.8. Block diagram of the dissolved oxygen concentration (DOC) control system

As shown in Figure 2.8, the DOC adaptive control system uses gain scheduling [120], but uses only the controller input/output signals for the development of the algorithm. The *Bioreactor* function block is modeled by using the validated models described in [25, 106, 103]. The DOC measurements were simulated by adding Gaussian noise:

$$c_{elm}(t_k) = c_{el}(t_k) + \sigma Randn \tag{2.38}$$

where c_{elm} is the measured value of DOC; σ is the standard deviation estimated from real measurements ($\sigma \cong 0.2\%$), $Randn$ is a sequence of normalized Gaussian random numbers. This noise level is considered as the maximal amplitude for these types of processes. In the gain-scheduling and PI-control algorithms, the time discretization step Δt = 0.18 s was used throughout the simulation experiments. An example on how to initialize *Matlab/Simulink* is presented in Appendix 1.

2.4. Gain-Scheduled control system for pH control

2.4.1. Development of adaptation algorithm for pH control

In fed-batch cultivation processes, the hydrogen-ions concentration can be modeled considering the influence of bacterial growth, as well as the dosing of acid and alkali solutions during pH control and dilution effects:

$$\frac{dC_{H+}}{dt} = (\alpha_1 \mu x + \alpha_2 x) + \frac{F_{pH}(C^0_{H+} - C_{H+})}{V} - \frac{F_s C_{H+}}{V} \tag{2.39}$$

where C^0_{H+} and C_{H+} are the concentrations of hydrogen-ions in the cultivation medium and in the alkali solution, respectively. Real and theoretical calculations of C^0_{H+} can differ and require model-based identification. x is the biomass concentration in the cultivation medium, g/l; μ is the biomass specific growth rate, 1/h; F_{pH} is the flow of the alkali solution for pH control, l/h; F_s is the flow of the feeding solution, l/h; V is the cultivation medium volume, l; α_1, α_2 are the model parameters to be identified from the experimental data. For practical implementation, F_{pH} and F_s can be calculated as:

$$F_{pH}(t_k) = \frac{F_{pH}(t_{k-1}) - F_{pH}(t_k)}{t_k - t_{k-1}} \tag{2.40}$$

$$F_s(t_k) = \frac{F_s(t_{k-1}) - F_s(t_k)}{t_k - t_{k-1}} \tag{2.41}$$

where $F_{pH}(t_k)$, $F_s(t_k)$, $F_{pH}(t_{k-1})$, $F_s(t_{k-1})$, t_k and t_{k-1} are taken from the experimental scale data of the alkali and substrate solutions. The initial value $C_{H^+}(0)$ is equal to 10^{-7} mol/l, and this level matches the pH level of 7. The linearization of Equation (2.39) around the process state point at time t_k with respect to the state (c) and the control (u) variables leads to the equation representing the C_{H^+} dynamics at time t_k:

$$\frac{d\Delta c}{dt} = -[\frac{\Delta c(F_{pH} + F_s)}{V}]_{t=t_k} + [\frac{\Delta u(C^0_{H^+} - C_{H^+})}{V}]_{t=t_k} \tag{2.42}$$

From Equation (2.42), the C_{H^+} dynamics can be represented by a first-order transfer function model:

$$G_{\frac{\Delta c}{\Delta u}}(s) = \frac{\Delta c(s)}{\Delta u(s)} = \frac{K_{pr}(t_k)}{T_{pr}(t_k)s + 1} \tag{2.43}$$

From this equation, the K_{pr} and T_{pr} controller parameters can be derived as:

$$K_{pr}(t_k) = [\frac{(C^0_{H^+} - C_{H^+})}{F_{pH} + F_s}]_{t=t_k} \tag{2.44}$$

$$T_{pr}(t_k) = [\frac{V}{F_{pH} + F_s}]_{t=t_k} \tag{2.45}$$

$K_{pr}(t_k)$ and $T_{pr}(t_k)$ are the process controller gain and the integration time constant at time point t_k, respectively, s is the Laplace operator.

As the time constants of the above control system elements are significantly smaller, compared with the time constant $T_{pr}(t_k)$, their influence on the controlled-process dynamics is taken into account by adding some time delay to the transfer function model (2.43). Therefore, the dynamics of the pH control process can be roughly represented by the FOPTD model:

$$G_{\frac{\Delta c}{\Delta u}}(s) = \frac{K_{pr}(t_k)}{T_{pr}(t_k)s + 1} \exp^{-\tau} \tag{2.46}$$

where τ, s is the time delay representing the influence of the dynamics of the control system elements. According to the PI controller tuning rules for static objects (Ziegler–Nichols, the internal model control (IMC), etc. [113]), the controller gain K_c is proportional to the ratio $Tpr/Kpr/\tau$, and the integration constant T_i is proportional to the resultant time constant T_{pr} [119]. Taking into account the functional

Relationships (2.44) and (2.45) and assuming that the controlled value of the pH during the cultivation process is close to the setpoint value, the character of relationships between the controller tuning parameters and the controller output and the setpoint signals can be estimated:

$$K_c(t_k) \sim T_{pr}/K_{pr}/\tau = \frac{1}{\tau}\frac{V}{C^0_{H^+} - C_{H^+}} \tag{2.47}$$

Based on Relationship (2.47), the gain scheduling algorithm for the controller gain adaptation takes the following form:

$$K_c(t_k) = \frac{K_{Kc}V}{C^0_{H^+}(t_k) - C_{H^+}(t_k)} \tag{2.48}$$

where $K_c \sim \frac{1}{\tau}$ is the coefficient for tuning the controller to obtain the desired performance of the control system (approximate values of the coefficient can be taken from the desired controller tuning rules).

The character of the relationship between the controller integration constant and the controller output is the following:

$$T_i \sim T_{pr} = \frac{V}{F_{pH} + F_s} \tag{2.49}$$

Based on Relationship (2.49), the gain scheduling algorithm for the controller integration time constant, the adaptation takes the following form:

$$T_i(t_k) = \frac{k_{Ti}V(t_k)}{(F_{pH}(t_k) + F_s(t_k))} \tag{2.50}$$

where k_{Ti} is the coefficient for tuning the controller to obtain the desired performance of the control system. Based on the available online measurements, the following control algorithms can be developed:

- A control algorithm where F_{pH}, F_s, and V are measured online.
- A control algorithm where F_{pH} and F_s are measured online, and a constant average value of V is used.
- A control algorithm where F_{pH} and V are measured online, and Fs is estimated as $F_s = kF_{pH}$,
- A control algorithm where only F_{pH} is measured online, V is considered constant and equal to the average value, and F_s is estimated as $F_s = kF_{pH}$,

where k is a tuning parameter. A diagram of the investigated adaptive pH control system realizing the adaptation algorithm with the controller output and input signals as gain scheduling variables is depicted in Figure 2.9.

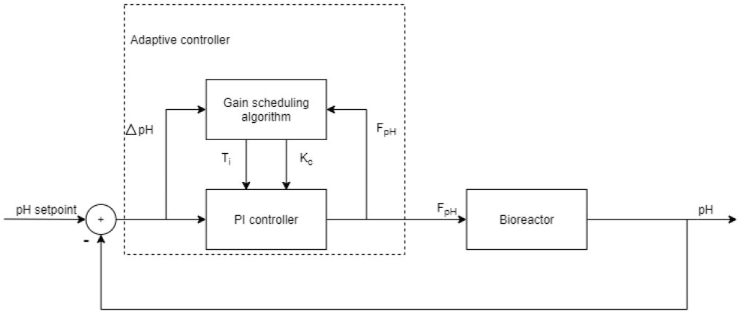


Figure 2.9. Block diagram of the pH adaptive control system

The adaptive control system uses gain scheduling [120], but uses only the controller input/output signals for the development of the algorithm. The *Bioreactor* function block is modeled by using the validated models described in [103].

2.5. Unified structure of adaptive system for pH control

2.5.1. Mathematical model for pH control simulation

In this simulation experiment, a mathematical model [103] was used to simulate the biotechnological process. The process P&ID is presented in Figure 2.10. This section focuses on the adaptive pH control loop marked as QC4.

The main process variable in the analysed system is the pH level of the medium. pH can be described as the concentration of free hydrogen-ions as:

$$pH = -log_{10} C_{H^+} \tag{2.51}$$

where C_{H^+} is the concentration of hydrogen-ions in the cultivation medium. The concentration of hydrogen-ions in a fed-batch cultivation process can be modeled considering the influence of bacterial growth, and the addition of acid and alkali solutions during the pH control and dilution effects:

$$\frac{dC_{H^+}}{dt} = (\alpha_1 \mu x + \alpha_2 x) + \frac{F_{pH}(C^0_{H^+} - C_{H^+})}{V} - \frac{F_s C_{H^+}}{V} \tag{2.52}$$

where $C^0_{H^+}$ is the concentration of hydrogen-ions in the alkali solution. This concentration can differ from the one calculated theoretically and is subject to model-based identification. x is the biomass concentration in the cultivation medium, g/l; μ is the biomass specific growth rate, 1/h; F_{pH} is the flow of the alkali solution for pH control,

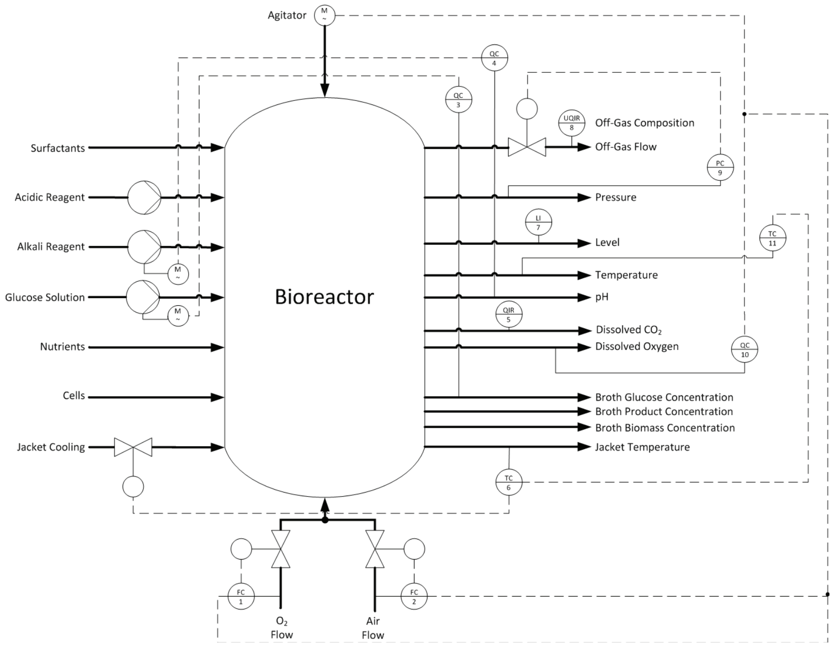


Figure 2.10. Basic control system loops for a typical microbial cultivation process [58]

l/h; F_s is the flow of the feeding solution, l/h; V is the cultivation medium volume, l; α_1, α_2 are the model parameters to be identified from the experimental data. For practical implementation, F_{pH} and F_s can be calculated as described in Equations (2.40)-(2.41).

The initial value $C_{H^+}(0)$ is equal to 10^{-7} mol/l, and this level corresponds to pH 7. The biomass growth in the fed-batch process can be modeled by means of the differential equation:

$$\frac{dx}{dt} = \mu x - \frac{F_s + F_{pH}}{V} x \tag{2.53}$$

OUR is modeled as follows:

$$OUR = \beta_1 \mu x V + \beta_2 x V \tag{2.54}$$

where β_1, β_2 are model parameters that need to be identified from the experimental data [103]. Additionally, for the simulation purposes, the model for the evaluation of the feeding solution flow is described as:

$$F_s = \frac{\mu_{set} x V}{Y_{xs} S_0} \tag{2.55}$$

where S_0 is the substrate concentration in the feed, g/l; Y_{xs} is the biomass/substrate yield coefficient, g/g. In this simulation experiment, SGR was held constant, $\mu = \mu_{set}$, since the real specific growth rate is not measured directly, and the process is controlled under substrate limitation conditions. The values of the model parameters from the previous experiments are given in Table 2.5. Model parameter identification is described in [103]. In this simulation experiment, only Phase 1 parameters are used.

Table 2.5. Values of model parameters [103]

Parameter	Value	Parameter	Value
Model parameter α_1	$0.422 \cdot 10^{-7}$ mol/g	Initial biomass concentration	2 g/l
Model parameter α_2	$0.011 \cdot 10^{-7}$ mol/g	Initial hydrogen-ions concentration	10^{-7} mol/l
Model parameter β_1	0.8646 g/g	Initial medium volume	5 l
Model parameter β_2	0.018 g/gh	Substrate concentration in feed S_0	450 g/l
Biomass/substrate yield coefficient	0.52 g/g	pH setpoint	7

As the real measurements of pH and OUR are corrupted by noise, the measurements in this simulation study were simulated by adding white Gaussian noise:

$$c_{elm}(k) = c_{el}(k) + \sigma Randn \tag{2.56}$$

where c_{elm} is the measured value of pH or OUR; σ is the standard deviation estimated from real measurements (σ= 0.1 % in the analyzed case), $Randn$ is a number from the Gaussian random numbers sequence with zero mean and unit variance; k denotes an index of a discrete measurement point. The time discretization step of the adaptation and the control algorithms is set to Δt = 0.18 s. In the simulation experiments, the time profile of the biomass specific growth rate variation, as presented in Figure 2.11, is chosen to simulate close to realistic operating conditions in the fed-batch cultivation process. In this study, the specific growth rate μ was maintained constant at 0.5 1/h. To simulate a system malfunction (a feeding pump failure or negative influence of the anti-foam agent addition), it was reduced to 0.1 1/h for 0.2 h every 2 hours starting from the 1^{st} hour of the cultivation process.

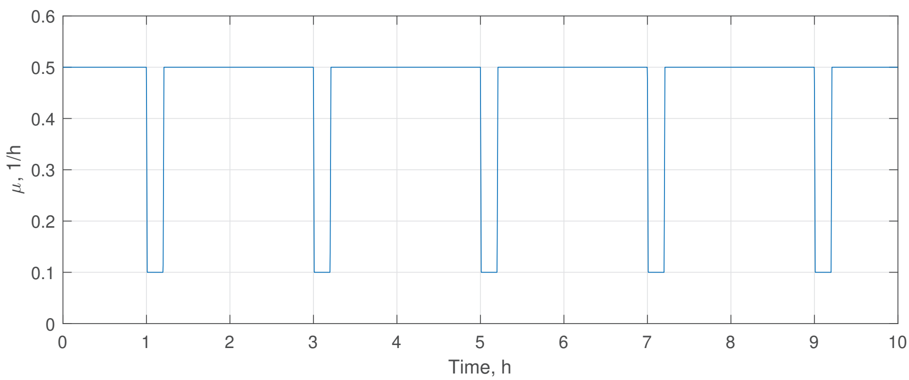


Figure 2.11. Specific growth rate μ trajectory during a simulation run

Since previously validated mathematical models which do not have any black box elements are used, no additional statistical analysis of the used models is performed.

2.5.2. Adaptation of PI controller parameters based on feedback signal statistical analysis

Previous studies of pH control systems in bioreactors have shown that, due to changes in the process dynamics, it would be appropriate to adapt the parameters of the PI controller, especially the integral time constant T_i, that has been proven to be the main tuning parameter that depends on the process load. The optimal value of the control parameter K_c depends only on the culture broth volume that does not change significantly during the process [103]. The adaptation of the controller parameter T_i is based on statistical analysis of the feedback signal of the system. In the controller adaptation algorithm, the average value of the error of the feedback signal c_{ave} is calculated online from a moving window:

$$c_{ave}(k) = \frac{1}{n} \sum_{i=k-n}^{k-1} c_{el}(i) \tag{2.57}$$

$$O_{ffset}(k) = c_{set} - c_{ave}(k) \tag{2.58}$$

where n is the moving window length that is subject to model-based optimization, -. c_{el} is the feedback signal value and c_{set} is the setpoint value,-. Additionally, the average absolute deviation is calculated from the feedback signal:

$$D_{absave}(k) = \frac{1}{n} \sum_{i=k-n}^{k-1} |c_{el}(i) - c_{ave}(k)| \tag{2.59}$$

The above presented statistical parameters are applied for online tuning of the controller integration constant T_i using the following rule:

IF $|O_{ffset}(k)| > O_{max}$ OR $D_{absave}(k) > D_{max}$
THEN $T_i(k) = T_i(k-1)(1 - a_1 O_{ffset}(k))$
ELSE $T_i(k) = T_i(k-1)$

where a_1, -, O_{max}, -, and D_{max}, -, are tuning parameters, and they are subject to model-based optimization. Controller gain K_c was not changed and was thus held constant during the controlled process. The block-diagram of the adaptive pH control system is presented in Figure 2.12.

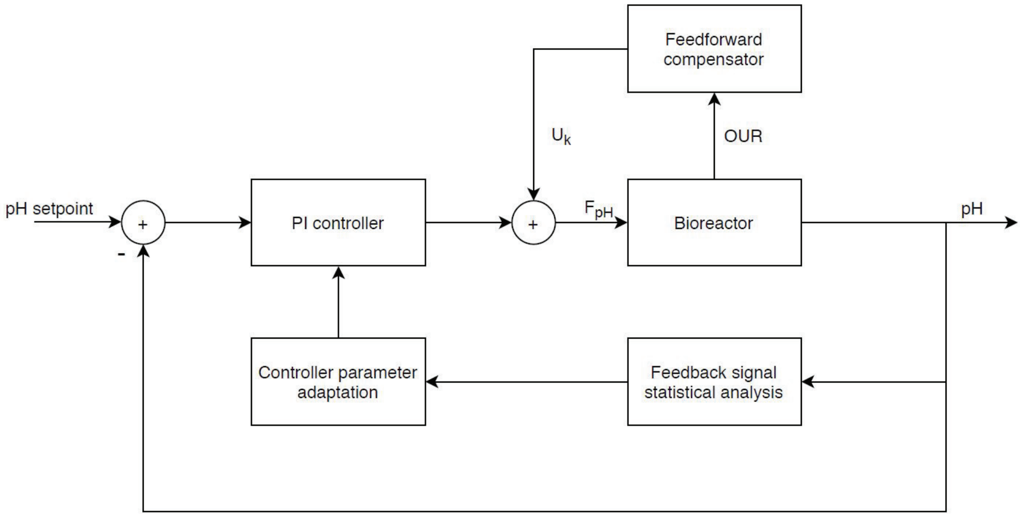


Figure 2.12. Block-diagram of the adaptive pH control system

The *Feedback signal statistical analysis* function block represents the statistical analysis performed on the controller's feedback signal originally introduced by R.Kalman [121]. To account for the variation of the bioprocess state, a feedforward compensator block [122] is implemented to support the adaptive PI controller which produces control action based on the OUR online estimation. Basic changes in the bioprocess state can be reflected by OUR that also correlates well with the dynamics of the system. This signal can be used as a basis for the feedforward compensation block in the proposed hybrid adaptive control. Currently, the industrial bioreactors can be equipped with off-gas composition (O_2, CO_2) and aeration gas flow rate measurement devices. This allows online estimation of the OUR signal. The feedforward compensator can be described as follows:

$$U_k = a_2 OUR \tag{2.60}$$

where a_2, l/g, is a tuning parameter that can be calculated from the other model parameters and manually tuned. The feedforward part defines the main part of the control signal, whereas the PI output signal is used for more precise tracking of the set pH value. The feedforward part of the control algorithm alone cannot assure sufficiently precise pH setpoint tracking under real conditions due to the occurring unpredictable metabolic shifts within the culture. The feedforward part is also inefficient because it cannot compensate large transients caused by the disturbances, even when used with a standard PI algorithm. There are various possible technological or specific growth control-related reasons why pH the setpoint can change or be manipulated, and system malfunctions could be one of them. It should be considered that the manipulation of the pH setpoint during the controlled process distorts the data in the moving window, and, therefore, the statistical parameter estimates as well. Since these values are used for the controller parameter adaptation, the adaptive control system based on the feedback signal statistical parameters is preferable to control the pH at a constant setpoint only. A suitable width of a moving window and the value of the coefficient a_1 in the tuning rule was determined from early simulation experiments by evaluating the IAE criterium with different parameter values. Different widths of the moving window n were tested. The optimal values of the tuning parameters are given in Table 2.6.

Table 2.6. Values of the control system tuning parameters

Parameter	Value
Controller gain K_c	6.6 h/l
Width of moving window n	2.7 s
Model parameter α_1	0.0205
Model parameter α_2	0.8174 l/g
Model parameter O_{max}	0.0001
Model parameter D_{max}	0.0015

2.6. DOC control in atypical cultivation processes

A major objective of fermentation is to obtain the highest possible amount of the product in a given volume and time frame. High cell densities are a prerequisite for high productivity [123]. The search for optimal conditions may lead to atypical cultivation processes where standard parameters (for example OUR) can change across a much wider range. An example here could be the DOC control where the following assumptions are made for the simulation of the DOC dynamics of an atypical cultivation process:

1. OUR in the atypical cultivation process is 3 times higher if compared with an ordinary cultivation process, therefore, $OUR_{atp}(t) = 3OUR(t)$;

2. Starting from the time point t = 4000 s, every 2000 s, some anti-foam agent is added into the bioreactor. This action extremely reduces the oxygen transfer capacity in the bioreactor for 30 seconds (parameter α is reduced by 70% for this time interval).

2.6.1. Mathematical model for DOC control simulation for atypical cultivation processes

In the simulation experiments, the controlled process was simulated by using the following state model described in [25, 84]:

$$\frac{dq}{dt} = \frac{1}{T_q}(q_{set} - q) \tag{2.61}$$

$$\frac{du}{dt} = \frac{1}{T_u}(u_{set} - u) \tag{2.62}$$

$$\frac{dc_a}{dt} = -OUR_{atp}\frac{c_a}{k_c + c_a} + \alpha u^{\beta} q^{\gamma}(\frac{y_{O_2}}{H} - c_a) \tag{2.63}$$

$$\frac{y_{O_2}}{dt} = \frac{q}{V}(\frac{1}{\epsilon} - 1)(0.21 - y_{O_2}) - \alpha u^{\beta} q^{\gamma}(\frac{1}{\epsilon} - 1)(\frac{y_{O_2}}{H} - c_a)v_{mol} \tag{2.64}$$

$$\frac{da_{el}}{dt} = \frac{1}{T_{el1}}(100\frac{c_a H}{0.21} - a_{el}) \tag{2.65}$$

$$\frac{dc_{el}}{dt} = \frac{1}{T_{el2}}(a_{el} - c_{el}) \tag{2.66}$$

where q_{set} is the set value of the air supply rate, lh^{-1}; u_{set} is the set value of the stirring speed (the control variable), h^{-1}; y_{O_2} is the portion of oxygen in exhaust gas, -; OUR_{atp} is the volumetric oxygen uptake rate, mmol l^{-1} h^{-1}, a_{el} is an auxiliary variable, %; c_{el} is the signal from the dissolved oxygen (DO) electrode, %; H is Henry's constant, l mmol^{-1}; V is the volume of the cultural liquid, l; v_{mol} is the volume of mmol of gas, l mmol^{-1}; T_q, T_u, T_{el1}, T_{el2} are time constants of the air supply system, the motor-stirrer system, and the DOC electrode, respectively, s; ϵ is gas holdup in the gas-liquid dispersion, -. The development of the above model is detailed in [25]. The values of the model parameters are given in Table 2.7. Since previously validated mathematical models that do not have any black box elements are being used, no additional statistical analysis of the used models is performed.

2.6.2. Adaptation algorithm development

In atypical cultivation processes, extremely large disturbances of the DOC may occur. Typically, the disturbances take place at time points when some anti-foam solution is added to the cultivation medium to avoid intensive foaming, which deteriorates important instrumentation of the bioreactor and spoils the cultivation process. Therefore, the foam reduction procedures are commonly used in atypical cultivation processes. This action extremely reduces the oxygen transfer capacity in the bioreactor. The set OUR profile is presented in Figure 2.13.

Table 2.7. DOC model parameter values and initial conditions of the state variables [25, 85]

	Model Parameters	
H = 0.7906 l mmol^{-1}	ϵ = 0.15	T_{el1} = 10 s
T_{el2} = 2 s	T_q = 2 s	T_u = 1 s
α = 0.8e-7	β = 2	γ = 0.2
k_c = 0.00265 mmol l^{-1}	v_{mol} = 0.0224 l mmol^{-1}	
	Initial Conditions	
$c_{el}(0)$ = 10%	$q(0)$ = 2 s^{-1}	$u(0)$ = 0.1 s^{-1}
$c_a(0)$ = 0.0266 mmol l^{-1}	$y_{O2}(0)$ = 0.2099	$a_{el}(0)$ = 10%

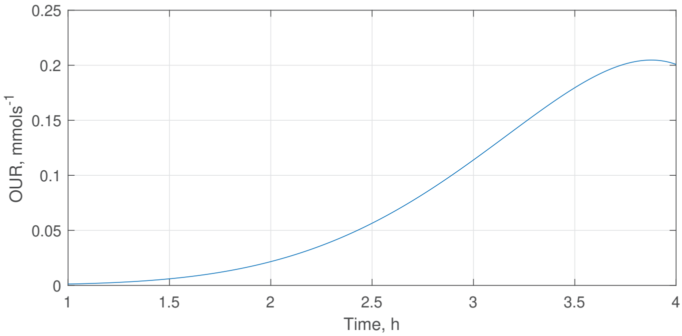


Figure 2.13. Selected OUR profile for biotechnological process simulation

Since the disturbances are directly related to OUR in the system, it was decided to use this parameter for the adaptation of the PI controller parameters. A 2^{nd} level polynomial of OUR was selected for testing:

$$K_p = a_1 + a_2 OUR + a_3 OUR^2 \tag{2.67}$$

$$T_i = b_1 + b_2 OUR + b_3 OUR^2 \tag{2.68}$$

The use of higher level polynomials was rejected due to the increased amount of the tuning parameters. The optimal parameters of the polynomial are determined by using the same genetic algorithm described in Section 2.1.2. Once again, the initial population was generated randomly. 20 simulation runs were performed to identify the optimal polynomial parameters. The parameters of the genetic algorithm are presented in Table 2.8.

Table 2.8. Genetic algorithm parameters

No. of Generations	Individuals in One Generation	Mutation Probability	Crossover Probability
130	300	0.1	0.9

Optimization was performed by minimizing the ISE value. After 40 generations, the ISE value did not change. The found parameters are presented in Table 2.9.

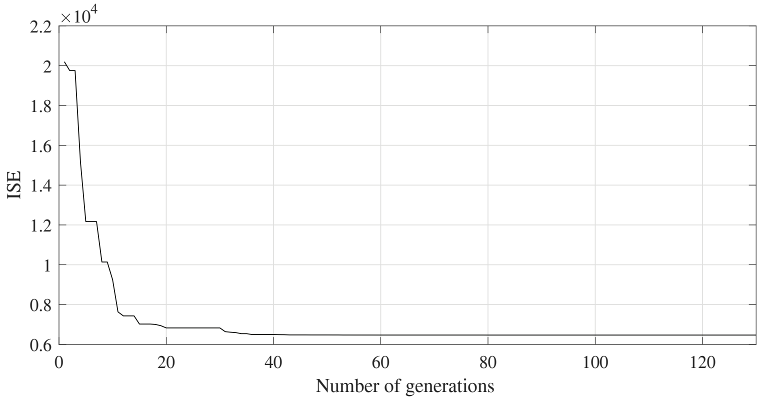


Figure 2.14. ISE criterion change during each generation of the genetic algorithm

Table 2.9. Optimal polynomial parameters presented as mean with 95% confidence interval

a_1	a_2	a_3	b_1	b_2	b_3	ISE
0.5142 [0.5118–0.5166]	-2.0258 [-2.0318–-2.0246]	4.48 [4.3118–4.5039]	78.3283 [77.6158–79.2186]	17.1954 [16.5216–17.5621]	1.4195 [1.4118–1.5109]	6468

2.7. Adaptation of substrate feeding profiles for fed-batch *E.coli* cultivation processes, based on an OUR and substrate feeding rate-based indicator

As stated in [124], the important issue in the designing of efficient substrate feeding strategies for fed-batch *E.coli* cultivation processes is to determine the reference substrate feeding profiles for the cultivation runs. For that, in the first step of the design procedure, a maximum glucose consumption rate in the biomass growth and target protein production phase must be determined by using data from unlimited growth cultivation experiments. Afterwards, the various scenarios for the glucose feeding rate need to be designed by setting different levels of the glucose consumption rate limitation during the cultivation process. Then, based on the experimental data of these cultivation runs, it is necessary to select a robust and efficient reference glucose feeding profile which then is recommended for implementation in recombinant protein production processes. The cultivation process quality is not influenced by temporary process disturbances due to the fact that the reference feeding profile is designed based on the proposed glucose limitation approach, thus making it robust enough to compensate for the disturbances. High amounts of glucose may accumulate in the bioreactor if the maximum specific glucose rate decreases by more than 5–8%, and that could be caused by a more significant disturbance in the system leading to an unstable and inefficient process. Modern industrial cultivation processes still cannot use online glucose concentration measurements for monitoring purposes. In such cases, the reference feeding profile needs to be modified based on the indirect monitoring of glucose accumulation in the cultivation medium. Figure 2.15 shows the proposed structure of the substrate feeding control system.

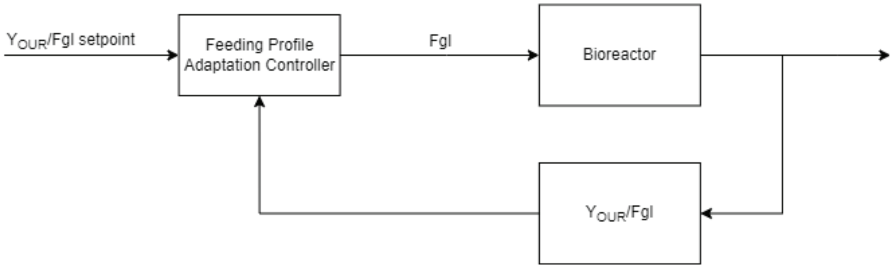


Figure 2.15. The structure of the proposed substrate feeding control system

The proposed system estimates the oxygen uptake yield on fed glucose in real time during the cultivation process. A moving window method with length n is used in the estimation where the oxygen uptake rate and the glucose feed rate are integrated thus determining the yield:

$$Y_{OUR/Fgl}(i) = (cOUR(i) - cOUR(i-n))/(S_0(cFgl(i) - cFgl(i-n))) \quad (2.69)$$

where $cOUR$ and $cFgl$ are the cumulative values of the oxygen uptake rate and the fed glucose. This yield is also identified for the reference cultivation process. Based on the $E.coli$ cultivation phase, the obtained yields change over time. Accumulation of glucose, caused by significant disturbances, decreases the oxygen uptake yield on the fed glucose.

The following equation is used to modify the reference glucose feeding rate based on the comparison of the real $Y_{OUR/Fgl}$ and the reference $Y_{OUR/Fgl_{reference}}$ yields. A velocity form PI algorithm is used to realize this modification:

$$E(i) = Y_{OUR/Fgl_{reference}}(i) - Y_{OUR/Fgl}(i) \quad (2.70)$$

$$U(i) = U(i-1) + K_c[1 + \frac{dt}{T_i}E(i) - E(i-1)] \quad (2.71)$$

$$Fgl(i) = Fgl_{reference}(i) - U(i) \quad (2.72)$$

$$dt = t(i) - t(i-1) \quad (2.73)$$

where $E(i)$ is the difference between the reference and the real yield, $U(i)$ is the controller's output, K_c, T_i is the controller's proportional gain and integral time, dt is the sampling time.

It is important to highlight that it is possible to use measurement devices in R&D and industrial bioreactors to analyze the composition of aeration gas, thus making the calculation of the oxygen uptake rate possible by using well known simple equations. This creates the possibility to use the proposed feeding profile adaptation algorithm in industrial bioreactors.

2.7.1. Mathematical model of recombinant *E.coli* cultivation process

Computed simulations were used to test the efficiency of the proposed substrate feeding control algorithm. The basis of the simulated system is a mathematical model of the recombinant *E.coli* cultivation process. Mass balance equations were used for the main model component, while the specific reaction rate expressions were defined by the biochemical conversion rates. Concentrations of biomass, x, g/kg, glucose, s, g/kg, target protein IFNa5 concentration (percentage in the cell as inclusion body), p_x, g/kg, as well as the culture medium weight, w, kg, are considered the main state variables in these simulation experiments.

$$\frac{dx}{dt} = \mu x - \frac{F}{w}x \tag{2.74}$$

$$\frac{ds}{dt} = -\sigma x - \frac{F}{w}s + \frac{F_s}{w}S_0 \tag{2.75}$$

$$\frac{dp_x}{dt} = IndK_p\mu \tag{2.76}$$

$$\frac{dw}{dt} = F - F_{smp} \tag{2.77}$$

The total mass flow to the bioreactor is calculated while taking into account sampling, F_{smp}, kg/h, and substrate feeding, F_s, kg/h, alkali buffer used for pH control, F_b, kg/h, loss of carbon due to CO_2 flux in the off-gas, F_{CO_2}, kg/h, and water evaporation through the vent line, F_e, kg/h. The biomass growth dynamics as well as various other important biotechnological process variables were considered for the simulation of mass flows:

$$F_b = Y_{XB}\mu x w \tag{2.78}$$

$$F_{CO_2} = -ROUR_v \tag{2.79}$$

$$F_e = -k_e w \tag{2.80}$$

$$F = F_s + F_b + F_{CO_2} + F_e \tag{2.81}$$

Oxygen consumption for the biomass growth and maintenance is taken into account while calculating OUR. The two-term relationship is used to calculate the per-unit weight and for the entire cultivation media weight:

$$OUR = Y_{ox}\mu x + m_{ox}x \tag{2.82}$$

$$OUR_v = OURw \tag{2.83}$$

The reaction rate limitation and inhibition is considered while modeling the specific reaction rates of the main components glucose, s, and biomass, x. The glucose specific consumption rate, σ, describes the substrate limitation and inhibitions related with the high biomass concentration and the concentration of the target protein synthesized inside the cell in the form of inclusion bodies:

$$\sigma = \sigma_{max}\frac{s}{K_s + s}\frac{K_x}{K_x + x}\frac{K_{p_x}}{K_{p_x} + p_x} \tag{2.84}$$

The substrate consumption and maintenance term, m, is used to model the biomass specific growth rate, μ. Acetate as well as other by-products of metabolic overflow are excreted due to high concentrations of glucose. In these situations, the biomass yield decreases, which leads to the formation of inhibiting by-products. Opportunely, in the analyzed cultivation process, the inhibition by metabolic overflow products was insignificant; therefore, the specific biomass growth rate was modeled based on the following equation:

$$\mu = (\sigma - m)Y_{xs}\frac{K_{YS}}{K_{YS} + s} \tag{2.85}$$

For the analyzed process formation of the target protein IFNa5 has been initiated by adding a rational amount of an inductor when the biomass concentration x exceeds 40g/kg ($Ind = 0$, if the biomass concentration is below 40g/kg, and $Ind = 1$, after it exceeds 40 g/kg). The target protein is formed in $E.coli$ cells in the form of inclusion bodies. The formation rate is then proportional to the specific growth rate, $Kp\mu$. The data from 15 fed-batch recombinant cultivation processes was used to identify the parameters of the described model equations. The results of the fermentations are presented in more detail in [107, 125]. The model development and the validated parameter values of Equations (2.74-2.85) are presented in Table 2.10 and further discussed in [107, 125].

Table 2.10. Model parameters [107, 125]

Model Parameter			
Y_{ox} = 0.8 gg^{-1}	m= 0.25 h^{-1}	Y_{xs} = 0.48 gg^{-1}	m_{ox}= 0.15 h^{-1}
σ_{max} = 1.822 h^{-1}	K_{p_x} =1.35 %	K_s = 0.02 gkg^{-1}	S_0 = 530 gkg^{-1}
K_p = 6.35 %	K_x =60 gkg^{-1}	F_{smp} = 0.01 kgh^{-1}	K_{YS} = 10 gkg^{-1}
Y_{XB} = 1.04e-3 gg^{-1}	K_e = 0.001 $h^{-1}kg^{-1}$	R = 1.05e-3 gg^{-1}	
Initial Conditions			
$w(0)$ = 3 kg	$x(0)$ = 0.15 gkg^{-1}	$s(0)$ = 2 gkg^{-1}	$p_x(0)$ = 0 gkg^{-1}

Since previously validated mathematical models that do not have any black box elements are used, no additional statistical analysis of the models in use is performed.

2.7.2. Experimental test of $Y_{OUR/Fgl}$ control algorithm

Three types of *Escherichia coli* cell-strain cultivations were performed, and their data was studied to verify the feeding profile adaptation estimates and determine their reliability and versatility. All three experiments were performed in a research and development laboratory at Kaunas University of Technology. The cell strain of *E. coli* BL21 (DE3) pET21-IFN-alfa-5 was cultivated in a 7 l bioreactor (BIO4, bioreactors.net). A *Siemens ET 200S* controller was used to control the process. The cultivation medium featured minimal mineral concentrations, including 46.55 g potassium dihydrogen phosphate, 14 g ammonium phosphate dibasic, 5.6 g citric acid monohydrate, 3 ml of concentrated antifoam, 35 g magnesium sulfate heptahydrate, and 105 g D (+) glucose monohydrate. The initial weight of the medium was 2 kg. The environmental parameters of the cultivation process remained constant throughout the experiment. The temperature was set to 37 °C, the DOT was set to 20% of air saturation, and the pH was maintained at pH 6.99 through the addition of NaOH(aq). The stirrer speed ranged from 100 to 720 rpm. The airflow scope ranged from 0.03 to 0.0567 g/s. During the cultivation process, pure oxygen flow from 0 to 7.5 l/min was being used to increase the oxygen transfer rate in the bioreactor. Based on the previously described recommendations, a reference feeding profile was determined and simulated by performing a safe limited growth experiment. The model parameters presented in Table 2.11 were used to simulate the reference feeding profile.

Table 2.11. Model parameters used to simulate the reference feeding profile for the experimental test run

Model Parameter			
Y_{ox} = 0.8 gg^{-1}	m= 0.25 h^{-1}	Y_{xs} = 0.48 gg^{-1}	m_{ox}= 0.15 h^{-1}
σ_{max} = 1.92 h^{-1}	K_{p_x} =4.95 %	K_s = 0.02 gkg^{-1}	S_0 = 530 gkg^{-1}
K_p = 6.35 %	K_x =60 gkg^{-1}	F_{smp} = 0.01 kgh^{-1}	K_{YS} = 10 gkg^{-1}
Y_{XB} = 1.04e-3 gg^{-1}	K_e = 0.001 h^{-1}kg^{-1}	R = 1.05e-3 gg^{-1}	
Initial Conditions			
$w(0)$ = 2 kg	$x(0)$ = 0.11 gkg^{-1}	$s(0)$ = 1.1 gkg^{-1}	$p_x(0)$ = 0 gkg^{-1}

Since previously validated mathematical models that do not have any black box elements, are used no additional statistical analysis of the models in use is performed.

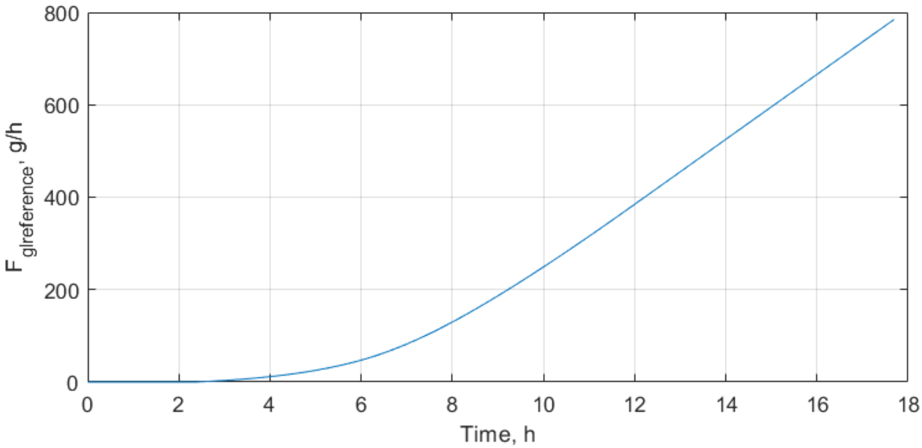


Figure 2.16. Selected reference feeding profile

To simulate a system disturbance and test the system's robustness, a heating malfunction was implemented, where the set temperature was reduced to 30 °C for 2 hours starting at t = 10 h. This disturbance was used in the two final experiment runs.

As described in Section 2.7, the feeding profile is adapted based on the $Y_{OUR/Fgl}$ ratio difference to the reference model:

$$E(i) = Y_{OUR/Fgl_{reference}}(i) - Y_{OUR/Fgl}(i) \tag{2.86}$$

$$U(i) = U(i-1) + K_c[1 + \frac{dt}{T_i}E(i) - E(i-1)] \tag{2.87}$$

$$Fgl(i) = Fgl_{reference}(i) - U(i) \tag{2.88}$$

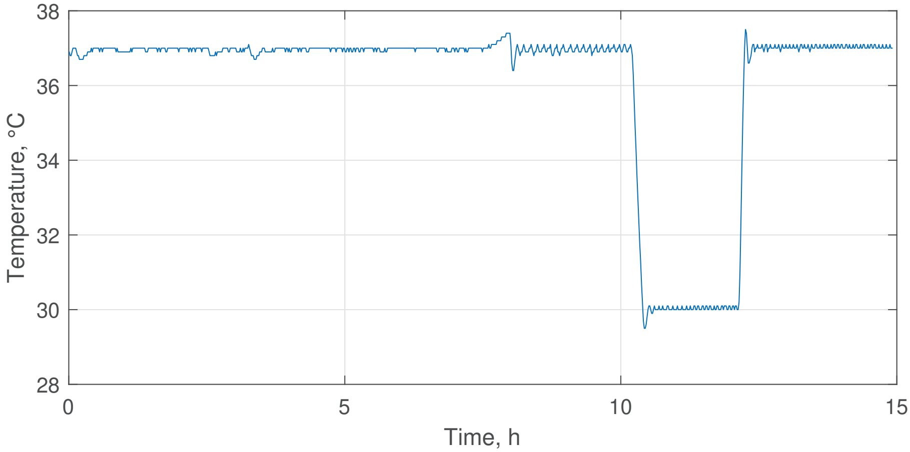


Figure 2.17. Set temperature change throughout the experiment

$$dt = t(i) - t(i-1) \tag{2.89}$$

The precision and reliability of the feeding profile adaptation algorithm were evaluated by comparing the offline calculations with the online values calculated during the cultivation experiment. To describe the feeding profile adaptation results, the indicators of the mean absolute error (MAE) and the root-mean-square error (RMSE) were applied. The MAE method evaluates the errors between the estimated and the observed $Y_{OUR/Fgl}$ values during the cultivation process. The MAE approach is defined as follows [126]:

$$MAE = \frac{\sum_{i=1}^{n} |y_m - y_{exp}|}{n} \tag{2.90}$$

where n is the number of data counts, and y_m is the estimation result compared to y_{exp}, which is the value determined through the cultivation process. The root-mean-square error represents the square root of the residuals of the differences between the modeled and observed values. The RMSE formula is as follows [126]:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (y_m - y_{exp})^2}{n}} \tag{2.91}$$

2.8. Conclusions of the chapter

1. Five different easy-to-implement PI controller parameter adaptation algorithms were developed and described. Fuzzy-based PI controller parameter adaptation focused on simple process knowledge transfer for PI controller parameter adaptation. The proposed model consists only of 3 membership functions and 4 rules, thus making it simple to implement and tune.

2. Gain scheduling-based models used controller input/output parameter values to calculate the PI controller parameters close to the real time without the need for any additional soft-sensors.

3. Two unified structure adaptation algorithms have been developed. PI controller parameters were adapted based on feedback signal statistical analysis and polynomial evaluation. These algorithms rely only on mathematical calculations, which makes them attractive for practical implementation.

4. A substrate feeding profile adaptation algorithm based on online $Y_{OUR/Fgl}$ yield profile adaptation has been developed and described. The algorithm has been tested in 3 experimental test runs at Kaunas University of Technology R&D laboratory.

3. PERFORMANCE EVALUATION OF THE DEVELOPED ALGORITHMS

3.1. Setpoint patterns

The performance of the developed algorithms was evaluated by using *Matlab/Simulink* simulations. To solve the differential equations, ODE functions and DEE Funktion blocks were used. Further detail on these blocks is presented in Appendix 1. To recreate close to realistic operating conditions, various setpoint and disturbance patterns were created and used in these simulation studies. The selected patterns consisted of several setpoints of different length and amplitude while also including maximal magnitude disturbances of the measured variable.

3.1.1. Specific growth rate pattern

An example of the setpoint profile of the SGR, as used for the performance evaluation of the fuzzy logic-based adaptive control algorithm (see Section 3.2), in a simulated process run is shown in Figure 3.1. The control quality was tested in various operation points of the process by changing the SGR setpoint every hour from 3 to 8 h. These switching points are characterized by different SGR setpoints and the accumulated biomass concentrations.

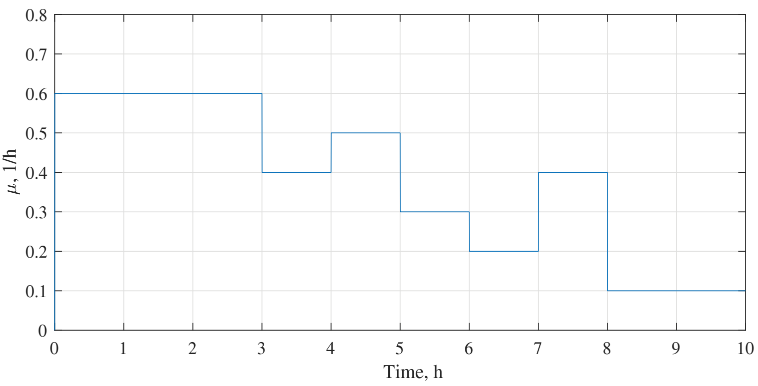


Figure 3.1. Selected specific growth rate setpoint profile for biotechnological process simulation

Additionally, in order to evaluate the controller's ability to remain stable and compensate for disturbances at various process phases, short pump faults were simulated at 4.5, 7.5 and 9 process hours, when the controller outputs value (reduction of the feeding pump performance) was reduced twice.

3.1.2. Dissolved oxygen concentration pattern

An example of the setpoint profile of the DOC that was being used to simulate close to realistic operation conditions in fed-batch cultivation processes is presented in Figure 3.2.

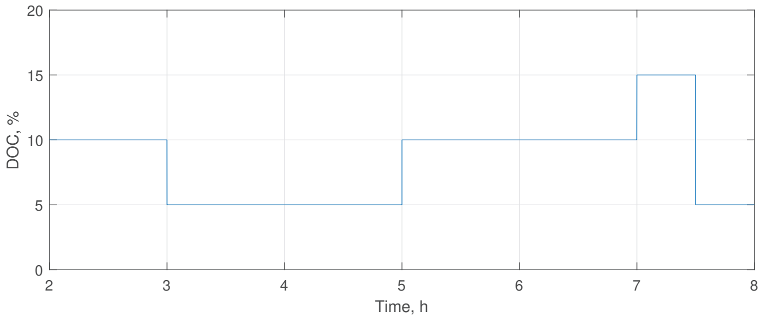


Figure 3.2. Selected DOC setpoint profile for biotechnological process simulation

During the simulation runs, DOC was held constant at setpoints varying from 5 to 15 percent. To simulate disturbances in the control system, air supply change was being used, and it is further described in Section 3.3.2.

3.1.3. pH pattern

During the simulations, pH was held at a constant setpoint of 7. To simulate disturbances in the system, the specific growth rate was reduced for various periods in time as described in Sections 2.5.1 and 3.5.1.

3.2. Performance evaluation of fuzzy logic-based adaptive SGR control

The performance evaluation of the created control algorithm is presented by using time profiles of the state variables and the calculation of the error value for the control algorithm. The ITAE criterion was selected to evaluate and compare the performance of the developed control algorithm. The adaptive PI controller with fuzzy-based parameter adaptation was compared with the gain scheduling (GS) and model-free adaptive (MFA) algorithms investigated in [58] using the same mathematical model of the biotechnological process (Equations 2.1-2.9). The GS method uses additional or already existing online measurements to adapt the controller parameters by using the derived functional relationships. The identification of such relationships requires in-depth analysis of the dynamic properties of the controlled process. The MFA control technique is an alternative data-based approach that does not require deep process knowledge needed for the creation of the mathematical model of the process. A two-layer neural network with an input layer that has a time-delayed sequence of the tracking errors [58] was used for comparison.

An example of the control performance of the developed PI controller with fuzzy-based parameter adaptation is presented in Figure 3.3.

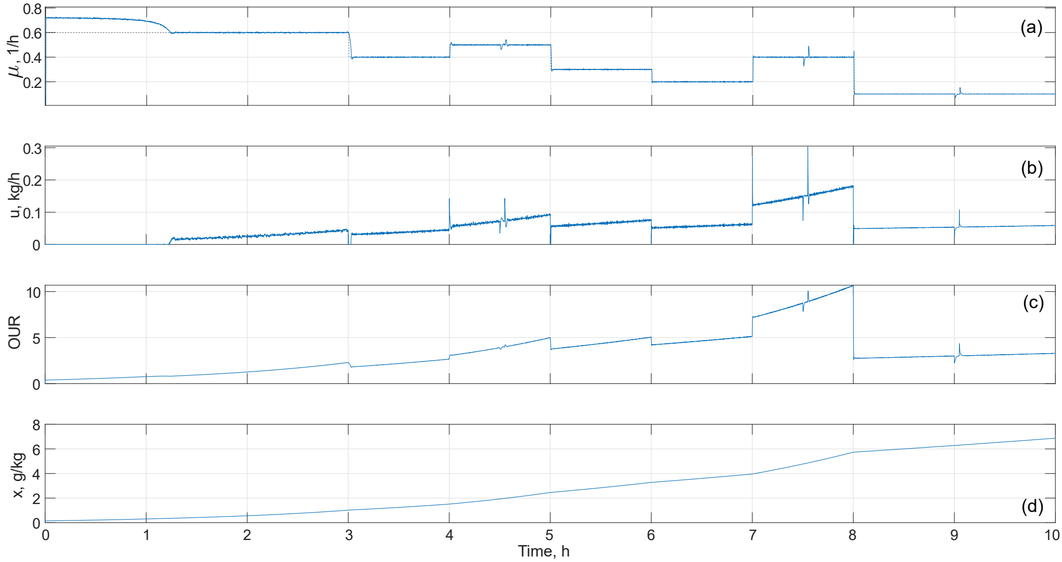


Figure 3.3. Specific growth rate (SGR) (system output variable) (a), substrate feeding rate (control variable) (b), oxygen uptake rate (OUR) (c), and biomass concentration (d) trajectories during the simulated process

During these simulation runs, the substrate feeding rate was manipulated to control the SGR. OUR and w measurments are needed for the fuzzy-based PI controller parameter adaptation. Model parameters for the simulation can be found in Table 2.1. The system remains stable across a wide setpoint range. Additive white Gaussian noise was used to corrupt the OUR signal during the simulations.

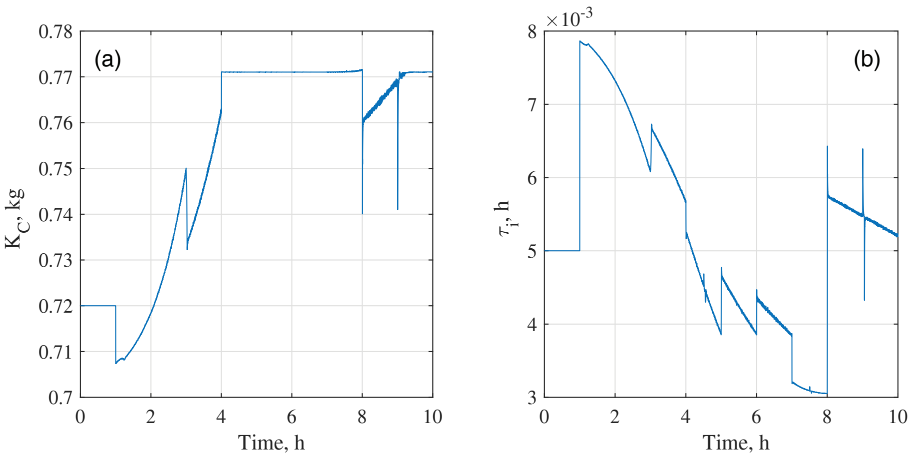


Figure 3.4. Development of PI controller tuning parameters during the simulated process, (a) the controller gain parameter K_c, (b) the integration time constant T_i

The change over time of the corresponding PI controller tuning parameter is presented in Figure 3.4. The controller gain parameter K_c (see Figure 3.4a) changes over time by approx. 10% only due to the fact that the parameter correlates with the culture broth weight w that increased only slightly during the simulated process. The integration time constant T_i (see Figure 3.4b) follows the changes of the OUR (see Figure 3.3c) profile, and, therefore, reflects the significantly varying dynamics of the process. The performance of all the presently mentioned models is summarized in Table 3.1. Here, one can see that the PI controller with fuzzy-based parameter adaptation was able to perform similar to the gain scheduling PI and MFA control algorithms in the investigated process with a given setpoint profile and acting disturbances.

Table 3.1. Performance comparison of adaptive SGR control algorithms

Control type	ITAE		
	MFA adaptation	GS adaptation	Fuzzy adaptation
Setpoint-tracking	0.6865	0.6592	0.6723
Disturbance rejection	0.3783	0.3962	0.3431
Setpoint-tracking and disturbance rejection	0.7598	0.7357	0.7349

All methods performed very similarly during setpoint tracking, while the fuzzy-based adaptation model performed the best during disturbance rejection when comparing with the other two models. The MFA model tends to perform better in phase II of the cultivation process, as shown in Figure 3.5.

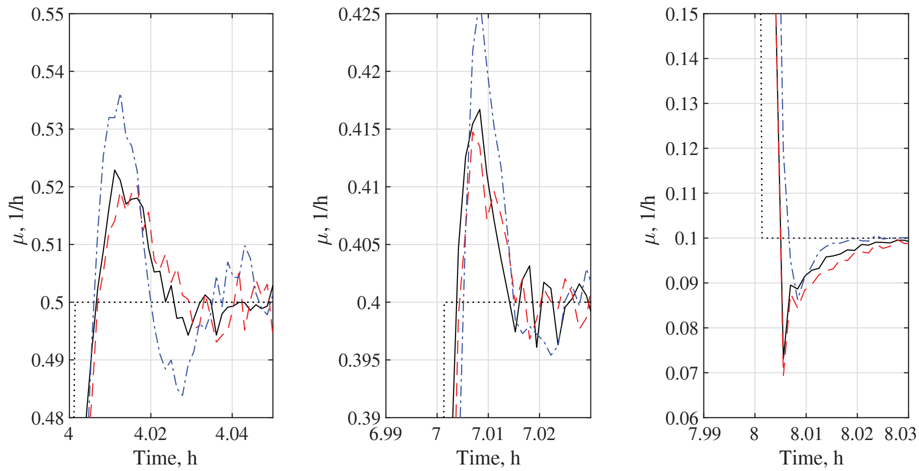


Figure 3.5. Transient processes of the SGR during a simulation run – setpoint change: PI controller with fuzzy-based adaptation (– solid line), PI controller with gain scheduling (- - dashed line), MFA (- . - dash-dot line)

When handling disturbances in the system, the PI controller with fuzzy-based parameter adaptation is able to outperform the GS and MFA algorithms in the first phase of the process; however, the MFA algorithm is again better suited for the second phase of the process as seen in Figure 3.6.

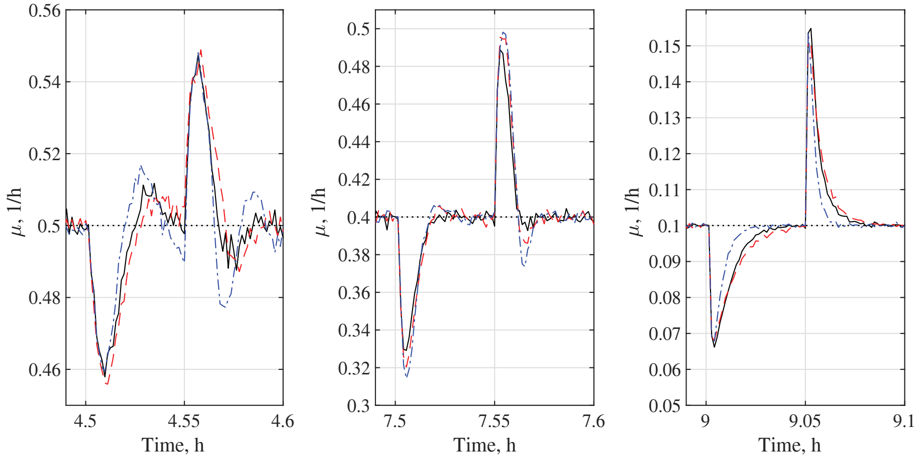


Figure 3.6. Transient processes of the specific growth rate during a simulation run – disturbance rejection when using: PI controller with fuzzy-based adaptation (– solid line), PI controller with gain scheduling (- - dashed line), MFA (- . - dash-dot line)

3.3. Performance evaluation of gain-scheduled dissolved oxygen control system

3.3.1. DOC setpoint-tracking performance

The bioprocess was simulated by numerically solving Equations (2.25)–(2.66) and by applying the controller parameter adaptation rules defined by Equations (2.23) and (2.24) for DOC control. Typical trajectories of the bioprocess variables are presented in Figure 3.7 for the case when the DOC setpoint tracking quality was investigated.

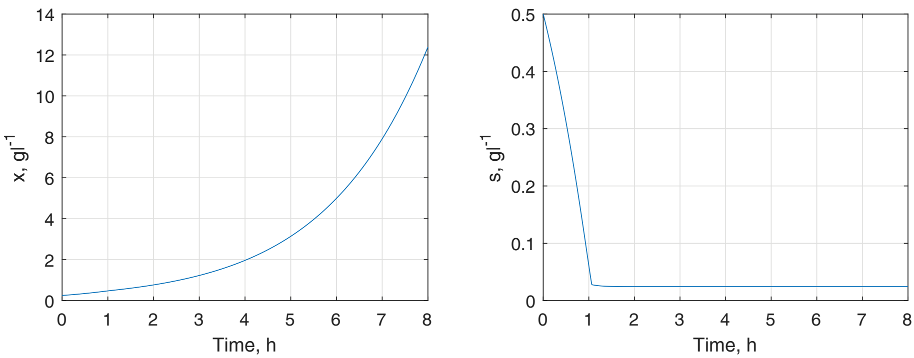


Figure 3.7. Trajectories of x (left), s (right) during a DOC setpoint tracking simulation run

After inoculation, the biomass x (Figure 3.7a) grows in the batch mode (until 1 h) while consuming a small initial amount of substrate s (Figure 3.7b). The culture broth volume V in the bioreactor (Figure 3.8c) changes due to the feeding flow of the substrate F_s (Figure 3.8d) which is initiated at the end of the batch phase (around 1 h).

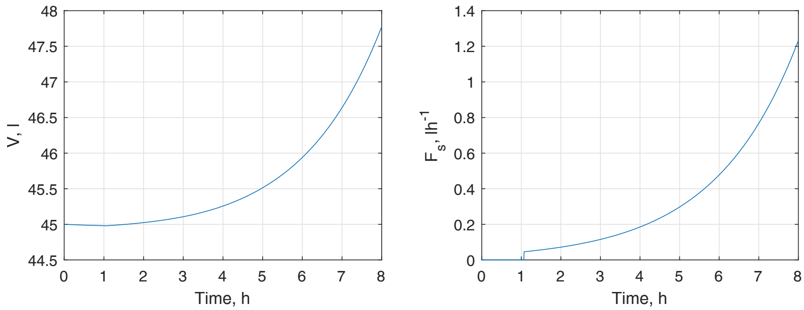


Figure 3.8. Trajectories of V (left), F_s (right) during a DOC setpoint tracking simulation run

The biomass specific growth rate depends on the actual substrate concentration and the DOC level (Equation (2.28), Figure 3.9e). Substrate oxidation and the subsequent biomass growth results in oxygen consumption, which is reflected by the oxygen uptake rate OUR (Figure 3.9f).

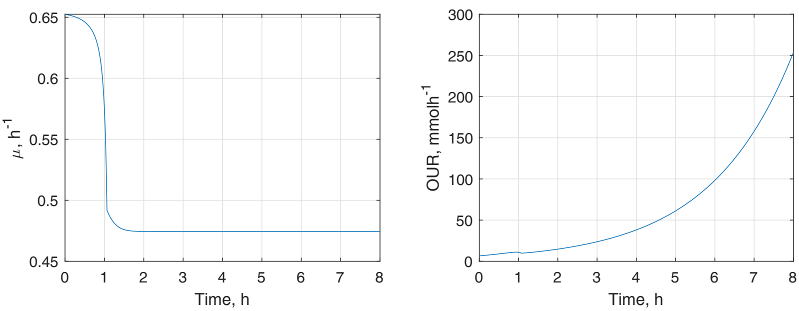


Figure 3.9. Trajectories of μ (left), OUR (right) during a DOC setpoint tracking simulation run

During the cultivation process, the DOC level is controlled by a PI controller. Both standard and gain-scheduled PI control systems were investigated and compared for the DOC control. First, the performance of the DOC adaptive control system was investigated for tracking the setpoint. In the simulation experiments, the time profile of the DOC setpoint change, as depicted in Figure 3.2, was selected for the simulation of close-to-realistic operating conditions in the fed-batch cultivation process. Stirring speed N was used as the control variable and was manipulated to control DOC. The model parameters for this simulation can be found in Tables 2.3 and 2.4.

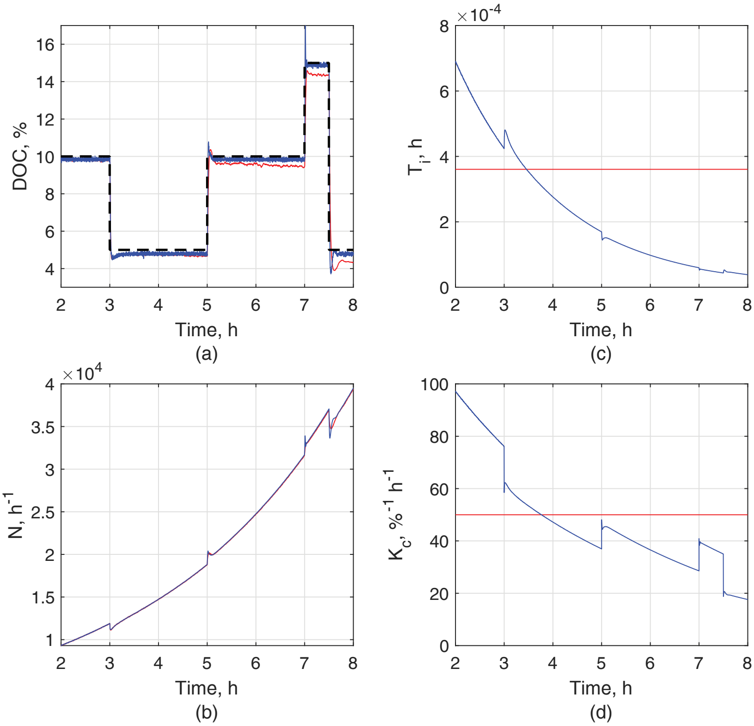


Figure 3.10. Trajectories of DOC (system output) (a), controller tuning parameter T_i (b), stirring speed N (control variable) (c), and controller tuning parameter K_c (d). Setpoint change: PI controller with fixed parameters (red), adaptive PI controller with gain scheduling (blue), DOC setpoint (black)

The performance of the gain-scheduled controller for step changes of the setpoint at 5 and 7.5 process hours is presented in Figure 3.11. The investigated adaptive control algorithm yields in a lower tracking error and a shorter rise time.

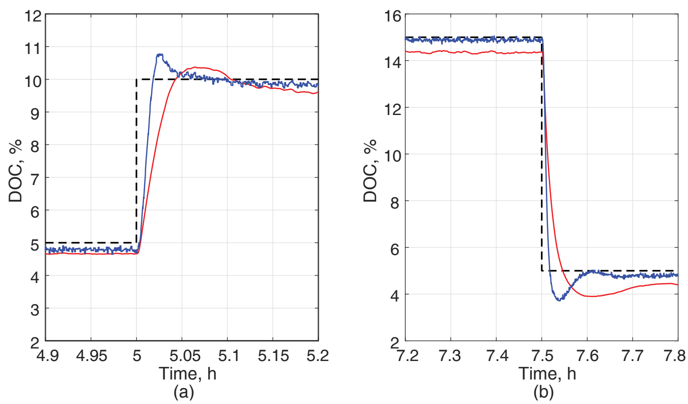


Figure 3.11. DOC responses (left) and (right) to setpoint change: PI controller with fixed parameters (red), adaptive PI controller with gain scheduling (blue), DOC setpoint (black)

3.3.2. DOC disturbance rejection performance

To evaluate the performance of disturbance rejection, the system was simulated at a constant setpoint of 10%. The air supply rate change was selected to simulate the disturbance. The change of the air supply rate is depicted in Figure 3.12.

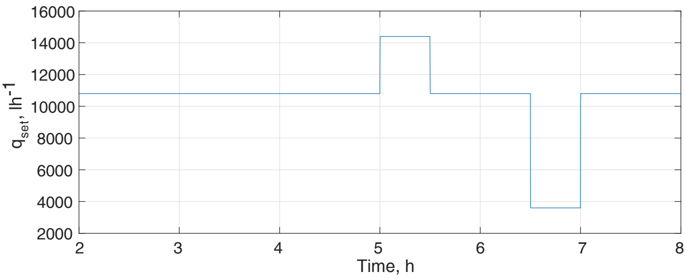


Figure 3.12. Air supply rate change during the simulation

The system's response and control performance are depicted in Figure 3.13a. The trajectory of the manipulated stirring speed N is presented in Figure 3.13b.

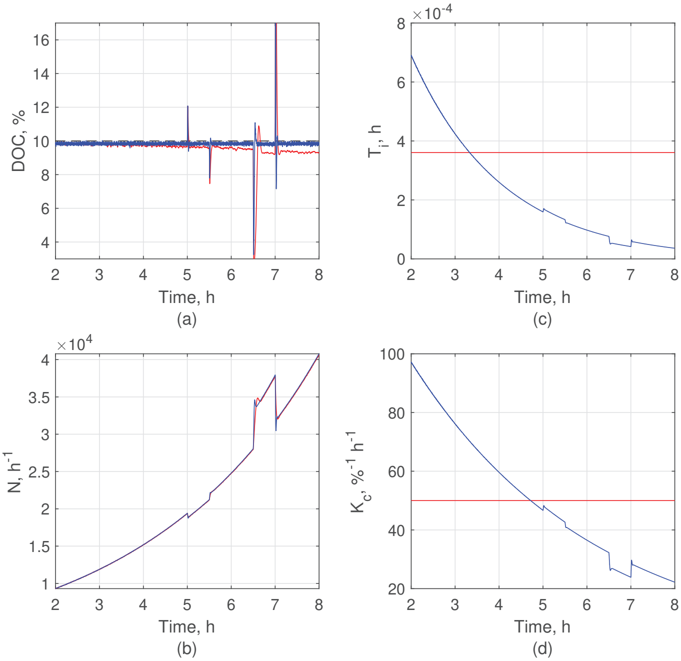


Figure 3.13. Trajectories of DOC (system output) (a), controller tuning parameter T_i (b), stirring speed N (control variable) (c), and controller tuning parameter K_c (d). Disturbance rejection when using: PI controller with fixed parameters (red), adaptive PI controller with gain scheduling (blue), DOC setpoint (black)

The performance of the gain-scheduled controller for disturbance compensation (the air supply rate step change from 10,800 lh^{-1} to 14,400 lh^{-1} occurring at t = 5.5 h, and from 14,400 to 3600 lh^{-1} occurring at t = 7 h) is presented in Figure 3.14. The adaptive control system yields a lower tracking error, and also reduces the overshoot.

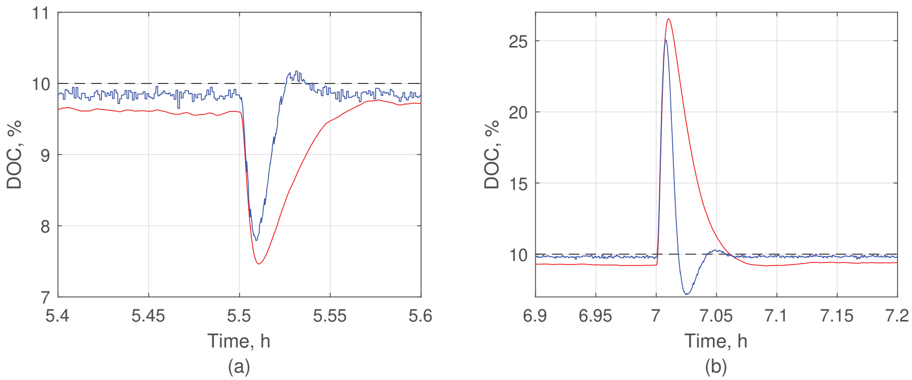


Figure 3.14. DOC disturbance compensation cases (left) and (right): PI controller with fixed parameters (red), adaptive PI controller with gain scheduling (blue), DOC setpoint (black)

The simulation results show that the gain scheduled PI controller ensures good control quality of DOC under extreme operating conditions (setpoint changes across a wide range and acting disturbances of maximal magnitude) and evidently outperforms the conventional PI controller. The integration time constant T_i and the controller gain K_c changed within a wide range, therefore reflecting the significantly varying dynamics of the process. Analysis of the simulation results shows that the adaptive system has reduced the mean absolute error by more than 2 times for the investigated control schemes. The rise time of the transient processes caused by the setpoint change was approx. 2 times shorter for the adaptive system (see Figure 3.11). However, both investigated systems yielded similar rise times in the case of disturbance rejection (see Figure 3.14). The control performance of the investigated systems is summarized in Table 3.2. The adaptive control algorithm outperforms the standard system by approx. 2 times in terms of the mean absolute error.

Table 3.2. Tuning parameters and MAE values for the investigated DOC control systems

Control type	Tuning parameters	Mean absolute error	
		Disturbance rejection	Setpoint tracking
Standard DOC	K_c = $50\%^{-1}h^{-1}$, T_i = 3e-4 h	0.166	0.071
Adaptive DOC	K_{Ti} = 0.6e5, K_{Kc} = 1.5e5	0.063	0.028

3.4. Performance evaluation of unified structure pH adaptive control system

The bioprocess was simulated by numerically solving Equations (2.52)-(2.55) and by applying the controller parameter adaptation based of feedback statistical analysis. pH control was selected to test the performance of the developed algorithm. Alkali solution feeding rate F_{pH} was selected as the control variable. Model parameters for this simulation can be found in Table 2.5.

The simulation results show that the investigated pH control system with the properly selected values of the tuning parameters provides reliable adaptation of the controller integration parameter T_i and stable performance. The simulation results, including the trajectories of OUR (Figure 3.15a), alkali solution feeding rate F_{pH} (control variable) (Figure 3.15c), and the adaptation of the controller tuning parameter T_i (Figure 3.15b) are shown in Figure 3.15.

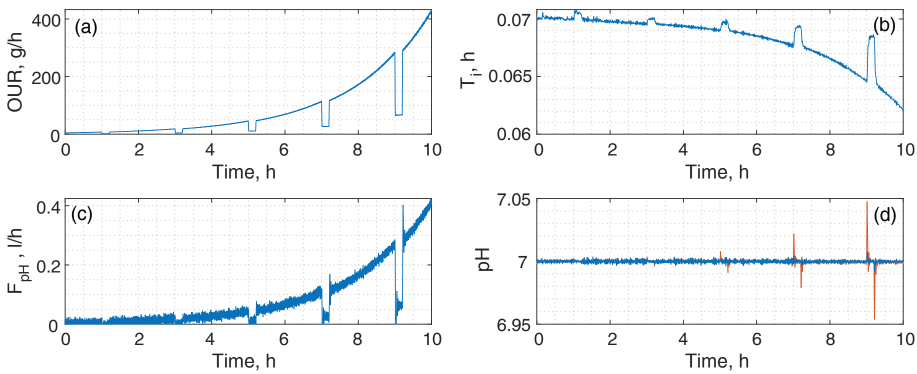


Figure 3.15. Adaptive control system output: (a) time profile of OUR, (b) adaptation of T_i controller parameter, (c) feeding solution rate F_{pH} (control variable), (d) comparison of the adaptive (blue) and standard (red) PI controller performance

For comparison, the performance of the standard PI control system with constant parameters (tuned to minimize the IAE criterion) is presented in Figure 3.15d. The adaptive control system tended to perform better at disturbance rejection, where the adaptive controller reduced the IAE criterion by almost 2.5 times (Table 3.3).

Table 3.3. Comparison of the adaptive and standard control system performance

Control type	**IAE** Standard PI	Adaptive PI
Disturbance rejection	0.0041	0.0017
Setpoint tracking and disturbance rejection	0.0126	0.0099

Analysis of the simulation results (Figures 3.16a and 3.16b) shows that, in the late phases of the cultivation process, the adaptive controller decreases the overshoot by approx. 80% in the adaptive system. Such a reduction in pH fluctuations is very important for cultivation process monitoring algorithms, where the rate of the carbon dioxide production rate, CPR, is used to monitor the state of the process, and the fluctuations in pH can cause drastic changes in CPR estimates. These disturbances may occur due to temporary substrate feeding disruption, faults of the bioreactor aeration or mixing systems, or sudden metabolic shifts. The presented results prove the efficiency of the pH adaptive control system and the potential of implementation in industrial controllers.

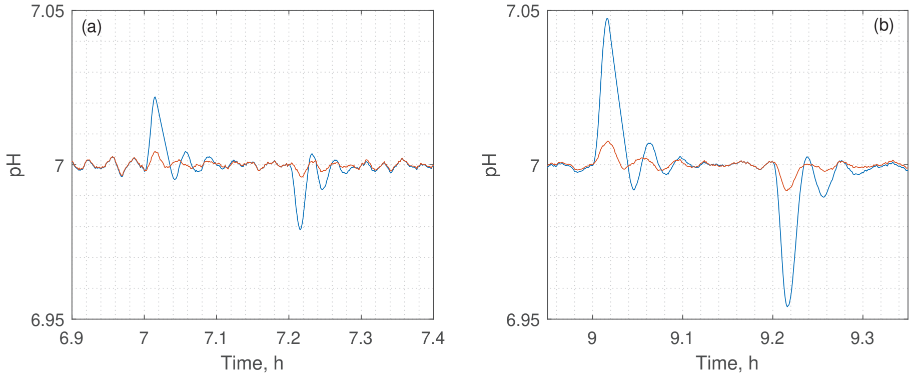


Figure 3.16. Transient processes of pH during a simulation run – disturbance rejection when using adaptive (red) and standard (blue) PI controller

3.5. Performance evaluation of gain scheduling based pH adaptive control system

3.5.1. pH control simulation and performance of the standard control system

The performance of the developed adaptive pH control system was investigated by using computer simulations with *Matlab/Simulink* software. In addition to Equation (2.52) for the balance of hydrogen-ions, the process model for the simulation includes the equations for biomass concentration and feeding rate profile [103], respectively:

$$\frac{dx}{dt} = \mu x - \frac{F_s + F_{pH}}{V}x \tag{3.1}$$

$$F_s = \frac{\mu_{set} x V}{Y_{xs} S_0} \tag{3.2}$$

where S_0 is the substrate concentration in feed, g/l; Y_{xs} is the biomass/substrate yield coefficient, g/g. In this simulation study, $\mu = \mu_{set}$ since the real specific growth rate is not measured directly, and the process is controlled under substrate limitation conditions. Table 3.4 consists of the model parameters and initial conditions for the simulated process. Model parameter identification is presented in [103].

Table 3.4. Values of the model parameters and initial values of the state variables [103]

Model parameters			
α_1 = 0.422·10^{-7} mol/g	α_2 = 0.011·10^{-7} mol/gh	Y_{xs} = 0.52 g/g	$pH_{setpoint}$ = 7
Initial conditions			
$x(0)$ = 2 g/l	$V(0)$ = 5 l	$C_{H^+}(0)$ =10^{-7} mol/l	S_0 = 450 g/l

As the real measurements of pH are corrupted by noise, the measurements in this simulation study were simulated by adding white Gaussian noise:

$$c_{elm}(k) = c_{el}(k) + \sigma Randn \tag{3.3}$$

where c_{elm} is the measured value of pH or OUR; σ is the standard deviation estimated from real measurements (σ= 0.1 % in the analysed case), $Randn$ is a number from Gaussian random numbers sequence with zero mean and unit variance; k denotes an index of discrete measurement point. The time discretization step of the adaptation and the control algorithms is set to Δt = 0.18 s.

In the simulation experiments, the time profile of the biomass specific growth rate variation, as presented in Figure 3.17, is chosen to simulate close to realistic operating conditions in the fed-batch cultivation process. In this simulation study, the specific growth rate μ was maintained constant at 0.5 1/h. To simulate a system malfunction (a feeding pump failure or negative influence of the anti-foam agent addition), it was reduced to 0.25 1/h for 1 h every 2 hours starting from the 2^{nd} hour of the cultivation process.

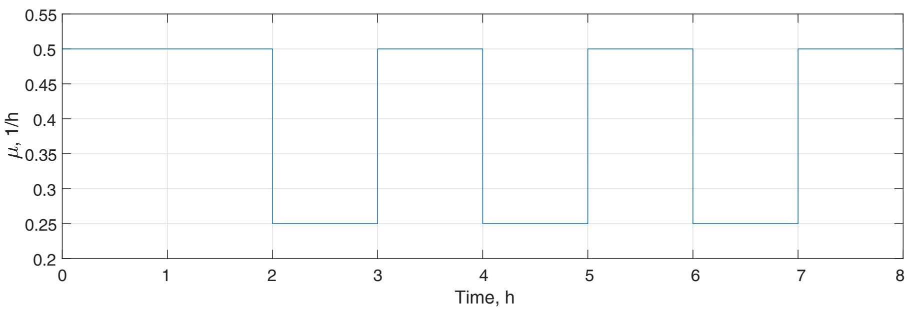


Figure 3.17. Specific growth rate μ trajectory during a simulation run

During these simulations, the alkali solution feeding rate F_{pH} was selected as the control variable. The performance of the pH adaptive control system was investigated for tracking the setpoint and the rejection of disturbances. From the preliminary experiments, the fixed tuning parameter values ($K_c = -3.3 \cdot 10^6$ h/l, T_i = 0.3 h) were determined that ensure a satisfactory control accuracy of pH and a stable signal (without significant fluctuations) of F_{pH} over time. The performance of such a standard PI control system (further referred to as System A) is shown in Figure 3.18.

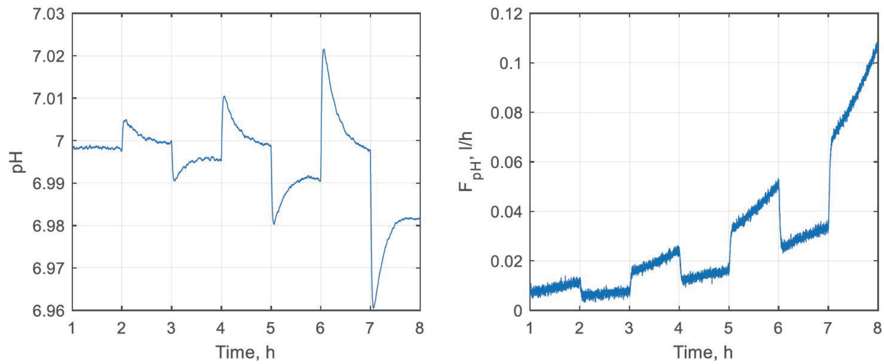


Figure 3.18. pH (system output) and F_{pH} (control variable) change over time during the fed-batch cultivation process using a standard PI controller

3.5.2. Performance of the adaptive control system with online-measured V and F_s values

As seen from Eqs. (2.48) and (2.50), the PI controller tuning parameters K_c and T_i depend on the controller input/output signals C_{H+} and F_{pH}, the substrate feeding rate F_s, and the culture medium volume V. Figure 3.19 depicts the performance of the developed adaptive control system, when the aforementioned signals are measured online, compared with a standard PI controller with fixed tuning parameters.

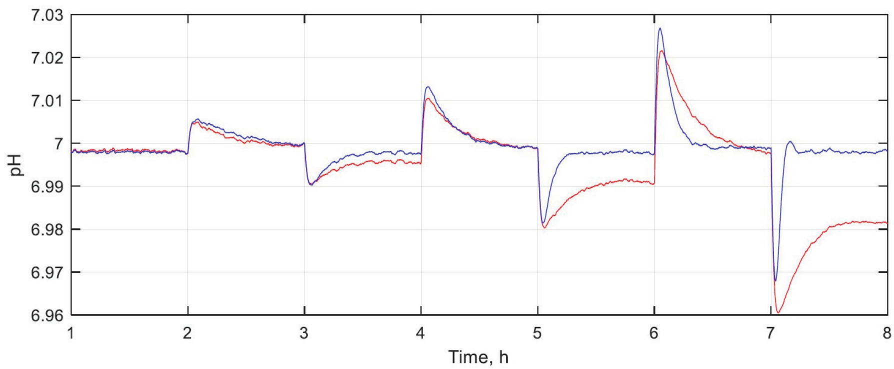


Figure 3.19. Adaptive control system output: comparison of the adaptive (blue) and standard (red) PI controller performance

The developed adaptive control system (further referred to as System B) outperformed the standard PI system at setpoint tracking. The change of the controller parameters can be seen in Figure 3.20.

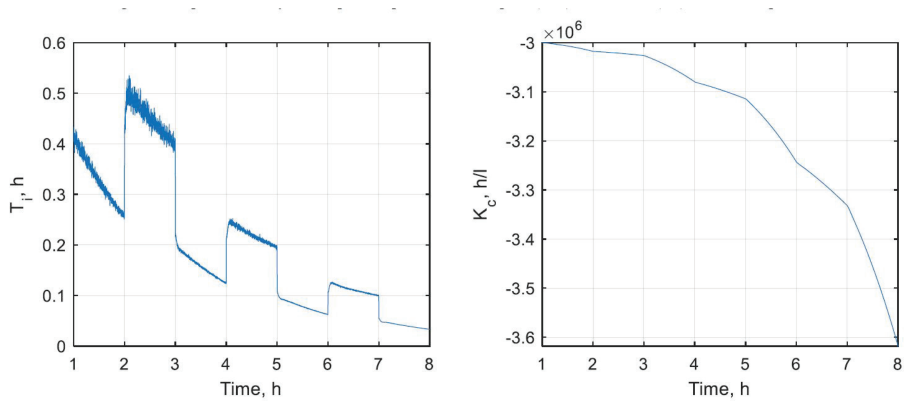


Figure 3.20. PI controller tuning parameter variation throughout the simulated process

The integration time constant T_i and the controller gain K_c changed in a wide range, therefore reflecting the significantly varying dynamics of the process. Analysis of the simulation results shows that the adaptive system has reduced the setpoint tracking error (ITAE) by almost 8 times.

3.5.3. Performance of the adaptive control system with online-measured F_s values

The developed adaptive algorithm requires the online measurement of the culture medium volume V and the substrate feeding rate F_s. To reduce the complexity of the system, the average medium volume can be used for the calculation of the gain scheduling parameters:

$$T_i = \frac{k_{Ti} V_{avg}}{F_{pH} + F_s} \tag{3.4}$$

$$K_c = \frac{K_{Kc} V_{avg}}{C^0_{H+} - C_{H+}} \tag{3.5}$$

where $V_{avg} = 5.5$ l, $k_{Ti} = 0.0035$ and $K_{Kc} = 0.1$. This slightly increases the ITAE value for setpoint tracking but slightly decreases it for disturbance rejection. The proposed adaptive system (further referred to as System C) handles disturbances better at the later stages of the process than the standard PI controller as shown in Figure 3.21.

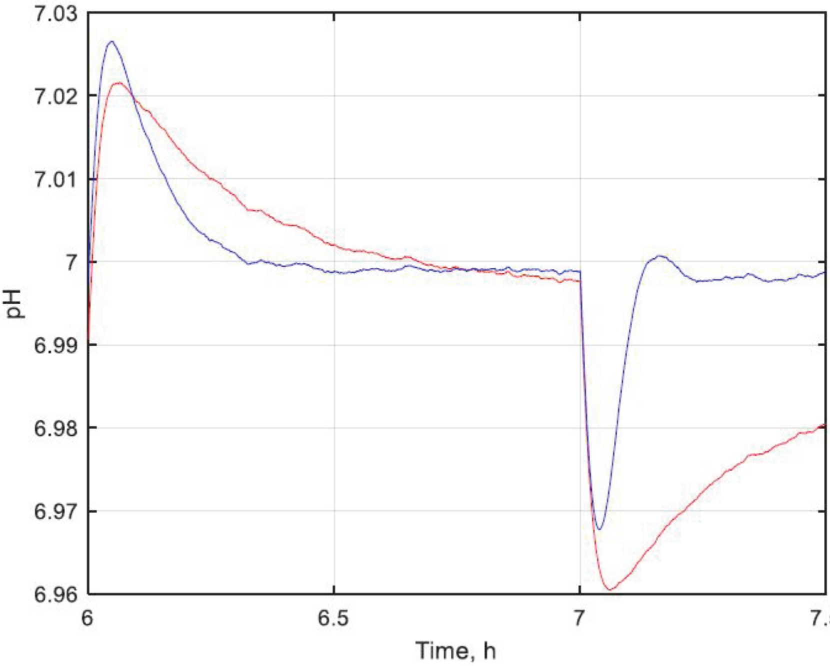


Figure 3.21. Comparison of the control performance of System (C) (blue), System A (red) at disturbance rejection

The ITAE criteria are reduced by 2.3 times compared with the standard PI controller with a fixed controller gain and the integration constant.

3.5.4. Performance of the adaptive control system while additionally measuring only medium volume

Since the substrate feeding rate F_s is approximately proportional to the pH controller's output (control variable F_{pH}) in the investigated substrate-limited cultivation processes, it can be assumed that:

$$F_s = kF_{pH} \tag{3.6}$$

where k is a tuning parameter which is subject to model-based identification. Such a simplified control system (further referred to as System D) performed similarly to the previously described System C, and it could be used as an alternative if the substrate feeding rate is not measured online. However, this model suffers from high T_i fluctuations at the beginning of the process, as shown in Figure 3.22.

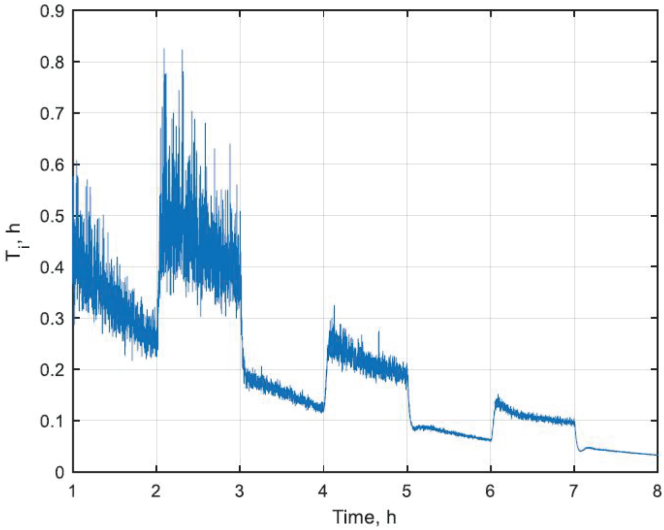


Figure 3.22. Controller parameter T_i change over time

3.5.5. Performance of the adaptive control system with controller input/output parameter based gain scheduling

The most attractive gain scheduling of PID (PI) controller parameters is based on only controller input/output signals, therefore not requiring additional measurement of the process variables for controller-parameter adaptation. Since the controller parameters K_c and T_i depend on the medium's volume V, alkali and substrate flows F_{pH}, F_s and Hydrogen-ion concentration C_{H^+} the input/output based adaptation can be described as:

$$T_i = \frac{K}{F_{pH}} \tag{3.7}$$

$$K_c = \frac{C}{C^0_{H^+} - C_{H^+}} \tag{3.8}$$

where $K = \frac{k_{Ti} V_{avg}}{k}$ and $C = K_{Kc} V_{avg}$ are the gain scheduling tuning parameters. The proposed adaptive systems tends to perform better than the standard system with fixed parameter values. However, simulation results show that the controller integration constant fluctuates intensively at the start of the process, as seen in Figure 3.23.

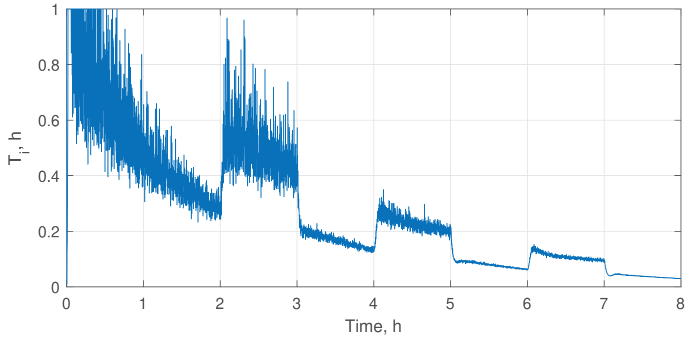


Figure 3.23. Controller parameter T_i change over time

To avoid this and increase the stability of the system, the adaptation of the controller parameters is initiated at $t = 3h$. The performance of the system (further referred as System E) is depicted in Figure 3.24

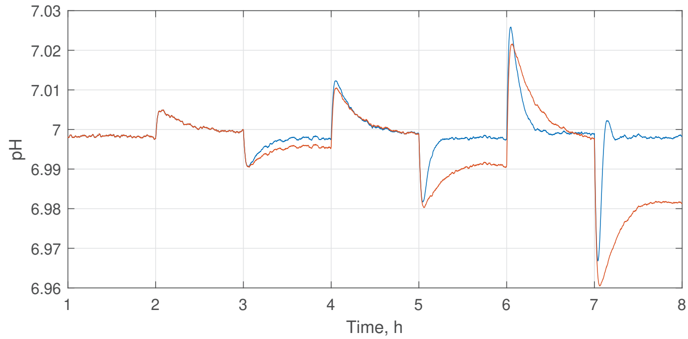


Figure 3.24. Adaptive control system output: comparison of the adaptive (blue) and standard (red) PI controller performance

A comparison of the ITAE values for the developed control systems is presented in Table 3.5.

Table 3.5. Overview of the developed pH control algorithms

Control type	ITAE	
	Disturbance rejection	Setpoint tracking
Standard control system A	0.1977	0.4567
Adaptive control system B	0.0866	0.0631
Adaptive control system C	0.0855	0.0632
Adaptive control system D	0.0859	0.0625
Adaptive control system E	0.0850	0.0615

3.6. Performance evaluation of DOC control in atypical cultivation process

The performance of the developed control system was evaluated by performing numerical simulations with *Matlab/Simulink*. The developed control system was compared with a standard control system with fixed PI controller parameters. The stirring speed N was used as the control variable to control DOC. Addition measurements of OUR were needed to calculate and adapt the PI controller tuning parameters. The modeling parameters can be found in Table 2.7. The adaptive control system tended to outperform the standard control system at disturbance rejection. The comparison of the DOC change in the simulated systems is presented in Figure 3.25.

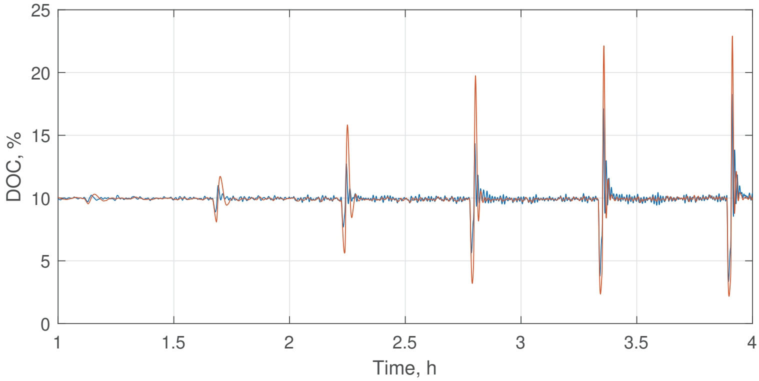


Figure 3.25. Adaptive control system output: comparison of the adaptive (blue) and standard (red) DOC controller performance

The standard PI controller was tuned by using the Ziegler-Nichols method. The adaptive control system reduced the ISE of the system and tended to perform better at disturbance rejection. The controller parameter adaptation is presented in Figure 3.26.

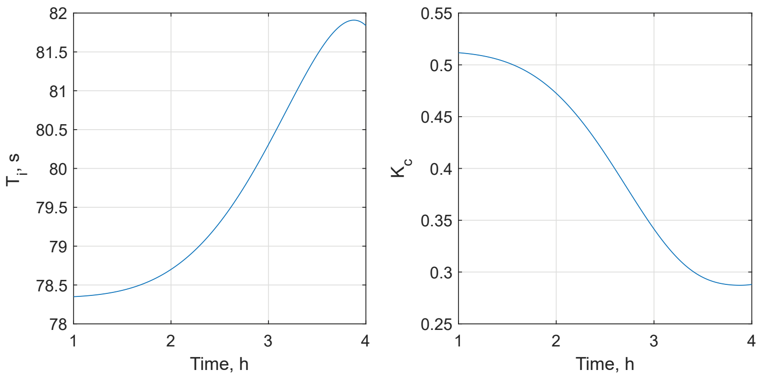


Figure 3.26. Controller parameter change over time

The adaptive control algorithm was able to reduce the ISE by almost 3 times compared with the standard controller with fixed tuning parameters (ISE = 18198). One can see that both controller parameters changed over time thus reflecting the time varying dynamics of the biotechnological process.

3.7. Substrate feeding profile adaptation, based on OUR and substrate feeding rate-based indicator; performance evaluation

3.7.1. Simulation results

The efficiency of the proposed substrate feeding control has been tested by using simulation studies with *Matlab*. The goal of the simulation is to imitate the behavior of a real cultivation process for producing interferon-alpha 5 (IFNa5) protein by the expression it in IFNa5 producing recombinant *E.coli* host [125, 107].

Simulated experiments with various glucose feeding scenarios were conducted. Different feeding profiles were generated where various glucose consumption rate limitations were realized by extending the cultivation duration in inverse proportion to the level of limitation. The total consumed glucose is then the same as in the unlimited experiment. Two phases can usually be described for feeding strategies in recombinant *E.coli* cultivation processes – the biomass growth phase and the recombinant protein production phase. In the first process phase, it is advisable to maintain the glucose consumption rate between 70 and 90$\%$ of its maximal value, thus making this a substrate-limited process. Such a feeding rate leads to a relatively high biomass growth rate but simultaneously ensures a low glucose concentration in the medium, enhances process repeatability, and mitigates the development of undesired metabolic by-products. Proper dissolved carbon dioxide concentration in culture media can be as well obtained by implementing restrictions on the maximum oxygen transfer rate in the bioreactor by selecting the right feeding profiles. In the second process phase, a recombinant protein is produced. The level of the glucose consumption rate and, as a result, the glucose feeding profile can be maintained between 50 and 90$\%$ of its maximum value. Therefore, the conditions for recombinant target protein production can be determined. Based on the results of these experiments, the reference glucose feeding profile, $Fgl_{reference}$, which ensures sufficient process robustness, repeatability, and high target protein production, should be selected. The oxygen uptake rate must be recorded since it is needed to determine the oxygen uptake yield profile on the fed glucose, $Y_{OUR/Fgl_{reference}}$.

In the real cultivation processes, it is recommended to use the selected reference glucose feeding profile. The process quality is in this case not impacted by typical short term disturbances while the designed feeding profile is being used. However, the accumulation of glucose caused by bigger disturbances should be avoided since the cultivation process may become unstable and inefficient. In Figure 3.27, the most important cultivation process variables for various substrate feeding regimes are pre-

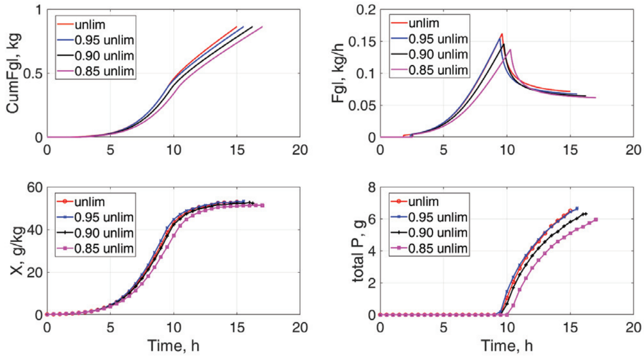


Figure 3.27. Dynamics of important cultivation process variables during various substrate feeding regimes

sented. For the proposed control system tests, the substrate feeding regime (reference process) was chosen, where the specific substrate consumption rate is 90% from the unlimited substrate consumption rate. In this case, the *E.coli* cultivation process is robust and, consequently, the typical short-term process disturbances (a short term decrease of σ by 50%) do not influence the cultivation process quality. The amount of the target protein P (P = $0.01{\cdot}p_x{\cdot}x{\cdot}w$) for such cultivation was approximately the same as in the reference process, P = 6.32 g. The typical cultivation process behavior for this case is presented in Figure 3.28.

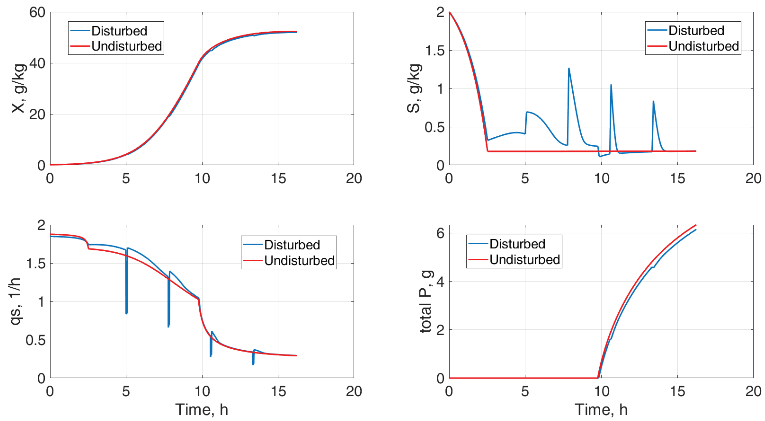


Figure 3.28. Dynamics of important process variables using the robust feeding profile with short term ($\sigma = 0.5\sigma$) and no disturbances

When process disturbances are of a higher intensity and duration, the quality of the cultivation process will probably be significantly reduced. The typical process behavior when 30% decrease in specific glucose consumption rate lasts 1 h is presented in Figure 3.29. This has caused the accumulation of substrate (glucose) in the medium and deterioration of the process quality with the decrease in the amount of target protein P, by 37%.

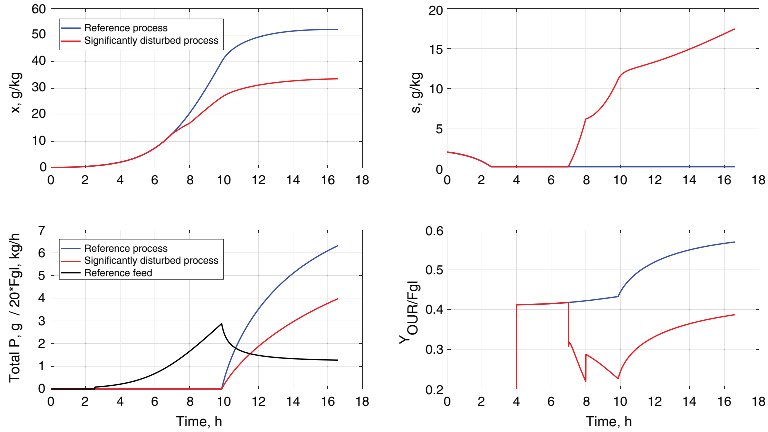


Figure 3.29. The behavior of process variables when 30% decrease in specific glucose consumption rate lasts for 1 hour (disturbance between 7-8 h)

Figure 3.30 shows another significantly disturbed cultivation process behavior. In this case, the maximum specific glucose consumption rate σ_{max} during the cultivation process was 5% smaller than in the reference process. This disturbance has also caused the significant accumulation of the substrate in the cultivation medium and the deterioration of the process quality with the decrease in the amount of the target protein P by 35.5%.

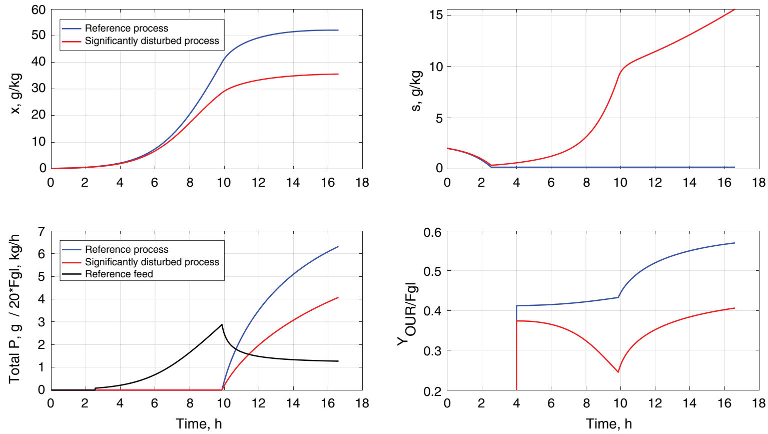


Figure 3.30. Behavior of process variables when specific glucose consumption rate σ_{max} is 5% smaller than in reference process (during all cultivation time)

To reduce the impact of these disturbances on the cultivation process quality, the proposed feed control system was used.

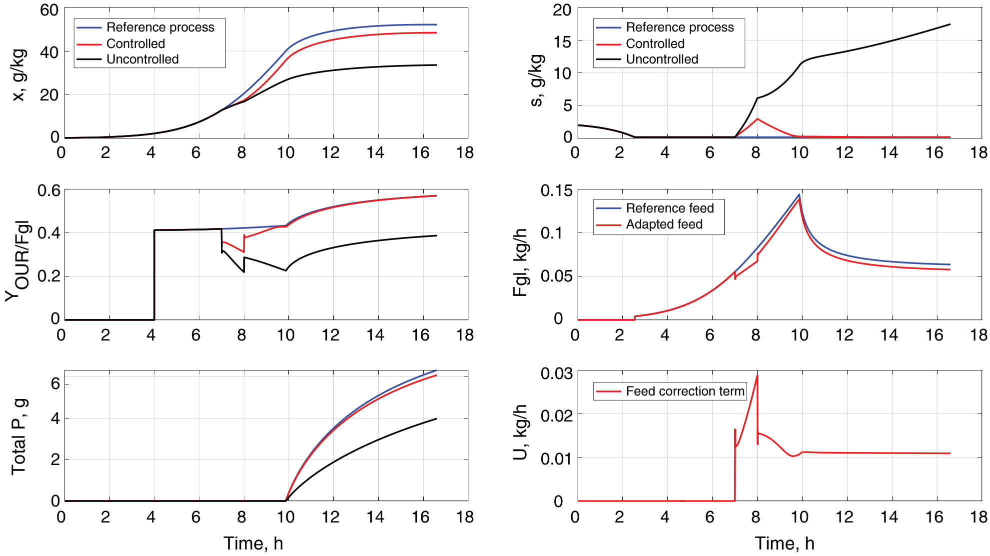


Figure 3.31. Test of proposed control system when 30% decrease in specific glucose consumption rate lasts 1 hour (cultivation time 7-8 h)

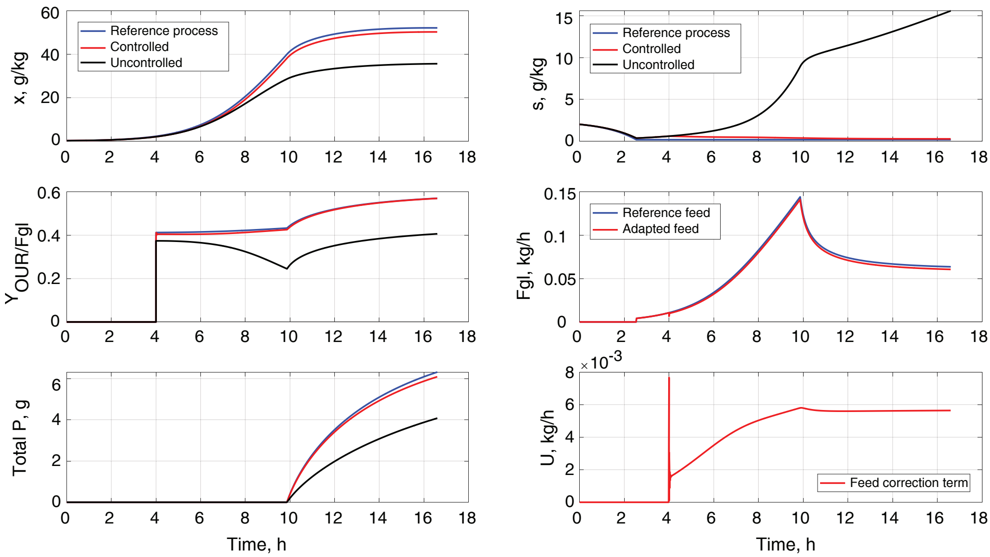


Figure 3.32. Test of proposed control system when specific glucose consumption rate σ_{max} is 5% smaller than in reference process (during entire cultivation process)

For that, indirect monitoring of the glucose accumulation in the cultivation medium and an online modification of the reference glucose feeding profile was realized. For the realization of this system, an online estimation of the oxygen uptake yield on the fed glucose, $Y_{OUR/Fgl}$, was performed by using the moving window approach (a 30-sec moving window) and compared with the reference yield, $Y_{OUR/Fgl_{reference}}$. Then, the velocity form of the PI controller was used for the modification of the reference feed profile.

The proposed feeding control system allowed to eliminate over-accumulation of glucose in the medium and improved the production of the target protein in the disturbed cultivation processes. The amount of the target protein P at the end of cultivation was only about 3.7% lower than in the reference process. Typical results of the application of this feeding profile adaptation schema are shown in Figure 3.31 and Figure 3.32, and summarized in Table 3.6.

Table 3.6. The efficiency of the proposed feed control system when the cultivation process is affected by significant disturbances

Process characteristics	Target protein, g without control system	Target protein, g with control system
Reference process	6.32	6.32
Disturbed process 1	3.98	6.08
Disturbed process 2	4.08	6.09

3.7.2. Experimental test results

To test the developed control algorithm, 3 experimental test runs were performed at KTU. During the first experimental test run, the reference feeding profile was determined by performing a safe limited growth experiment. During this experiment, the OUR and substrate feeding rate Fgl values were monitored to determine the previously described $Y_{OUR/Fgl}$ yield profile. This yield profile was then used by the control algorithm as the setpoint value, and, with the help of the velocity form PI control algorithm, adopted the substrate feeding rate. In the later experiments, a temperature disturbance was implemented. Model parameters for the simulation can be found in Table 2.11. A comparison of the estimated and experimental yield profile is presented in Figure 3.33.

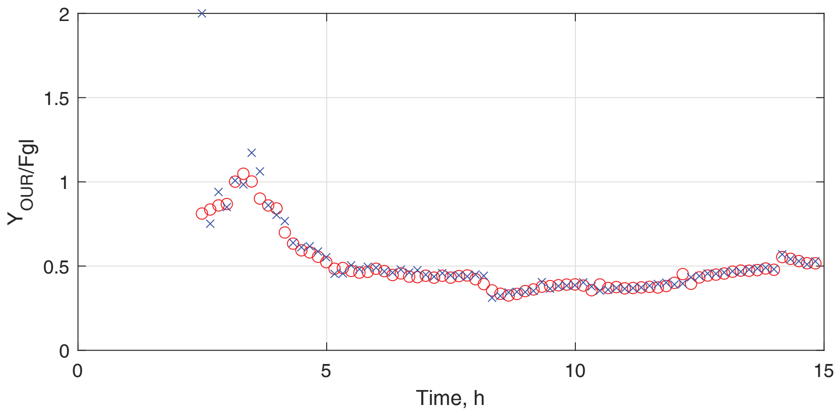


Figure 3.33. Estimated and online measured $Y_{OUR/Fgl}$. Estimated data is represented by red circles. Erperimental data is represented by blue marks

The feeding profile adaptation results for the three different experiments are shown below.

Table 3.7. Mean absolute error and root mean square error comparison for online and offline $Y_{OUR/Fgl}$ during all experimental runs

Experiment Number	Mean Absolute Error	Root Mean Square Error
1	0.0110	0.0230
2	0.0230	0.0580
3	0.0220	0.0790

The overall average MAE of the feeding profile adaptation algorithm was 0.018, and the overall average RMSE of the SGR estimation was 0.053. These results show that this approach is acceptable for fed-batch cultivation processes with *E. coli* cells.

Comparison of the previously calculated feeding profile, the modeled profile, and the adapted feeding profile after the test run is presented in Figure 3.34.

It can be seen that the adaptation algorithm adapts the pre-set feeding profile to cope with the biotechnological parameter changes throughout the cultivation. During one of the experimental runs, the adaptation algorithm was able to compensate the feeding loses which were caused by a broken tube. This can be considered as an advantage for the practical implementation of this control method. The simulation and experimental test results fulfill the minimal requirement for model verification. Due to the high costs of the experimental runs, the model validation shall be performed in future studies.

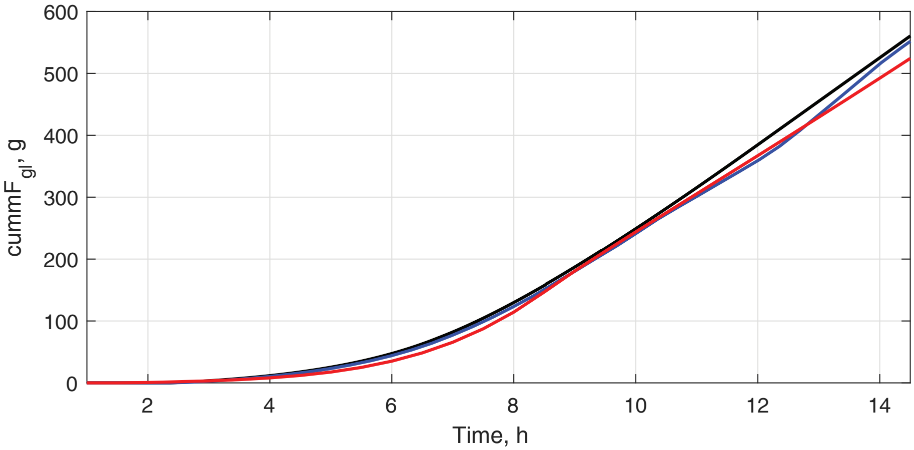


Figure 3.34. Adaptation of the feeding profile. The black line represents the reference profile. Adapted feeding profile is represented by the blue line for the experimental test run, and the red line for the simulated run

3.8. Conclusions of the chapter

1. In general, the investigated control techniques perform similarly when considering the whole process duration. The type of controller that should be selected, therefore, depends on the existing knowledge of the process and the control type. The adaptive PI controller with fuzzy-based parameter adaptation demonstrates advantages over the gain scheduling PI and model-free adaptive algorithms when compensating for the SGR deviations that could be caused by various process disturbances or equipment malfunctions. The gain scheduling PI approach requires deeper process knowledge and analytical skills to develop the adaptation model. This control type performs best when no disturbances or malfunctions are present in the system. The MFA controller tends to perform best during the second phase of the process. During this phase, the production of the recombinant protein occurs, and the SGR is lower. The simulation experiments also revealed that the fuzzy-based control system was stable within the investigated ranges. Considering practical implementation, the developed adaptive control algorithm requires off-gas analysers/flow meters and continuous-flow peristaltic pumps, so that the applicable sampling interval of the closed-loop control system is not exceeded. This can occur due to the time constants of the measuring devices or gas transport delays. The observed results show that, with regard to control performance, the described controllers are suitable for SGR control in fed-batch biotechnological processes if the substrate feeding rate manipulation and limitation approach is used. Taking into account the time needed for controller design and tuning, the fuzzy-based PI controller is

suitable for practical applications whenever expert knowledge is available. The developed controller also tended to deliver better control quality when handling disturbances and setpoint-tracking in the first phase of the process. Hence, the fuzzy-based control algorithm is an attractive alternative approach to control the specific growth rate in fed-batch recombinant protein production processes.

2. A simple adaptive control system for the setpoint control and disturbance rejection of dissolved oxygen concentration and pH has been proposed, in which the gain scheduling of the PID (PI) controller is based on the controller input/output signals only, and, therefore, does not require additional online measurements of process variables for the development of gain scheduling algorithms. The realization of the proposed system does not depend on the instrumentation level of the bioreactor and is attractive for practical application. The controller input/output-based gain scheduling algorithms were developed for setpoint tracking and disturbance rejection during DOC and pH control for a bioreactor operating in the fed-batch mode. The performance of the gain-scheduled PI controller under extreme operating conditions has been investigated by computer simulation. The results demonstrate obvious advantages of the proposed control system compared to the conventional PI control systems.

3. A simple pH control algorithm has been proposed. The developed adaptive PI control algorithm has been investigated for controlling pH in a fed-batch fermentation process. The control system remained stable and showed improvements of the pH control accuracy in comparison with the standard PI control algorithm with fixed controller parameters. The adaptive control algorithm based on the statistical analysis of the feedback signal can be easily implemented in many commercial controllers. It can be applied for controlling pH at a steady setpoint in standard fed-batch fermentation processes under ordinary conditions. The application of the proposed adaptive control algorithm with a feedforward compensator can be extended to control the other basic process variables of biotechnological cultivation processes.

4. DOC control in atypical cultivation processes has been tested. Controller parameters have been calculated and adapted based a second degree polynomial expression of OUR measurements. The adaptive controller outperformed the standard PI controller and is attractive for practical implementation due to its simplicity; however, off-gas analyzers are needed for practical implementation.

5. An adaptation algorithm for glucose feeding profile adaptation has been proposed. The simulation tests have shown that, when the glucose feeding profiles are designed with a slight growth limitation on the substrate, the small disturbances in the cultivation process do not influence the efficiency of the process,

and the total amount of the target protein is similar to that in the reference process. When the cultivation process is affected by significant disturbances or hardware failures, a feed control algorithm is required to ensure the efficiency of the process. The proposed feed control algorithm modifies the reference feeding profile when the process disturbances occur, and glucose begins to accumulate unpredictably in the cultivation medium. Tests performed by using the 'virtual bioreactor' approach and tests performed in the real cultivation processes have shown the performance and efficiency of the proposed control algorithm, thereby making it attractive for practical implementation.

4. CONCLUSIONS

1. The developed fuzzy logic-based adaptive control algorithm outperforms other adaptive techniques when compensating for deviations. The ITAE value has been reduced by 9% for disturbance rejection and 0.11% for setpoint tracking and disturbance rejection, thus making this algorithm suitable for fed-batch biotechnological process control.

2. The mean absolute error was reduced by more than 60% for disturbance rejection and setpoint tracking for both pH and DOC control when using the developed adaptive control algorithms based on gain scheduling. By using only controller input/output variables, no additional online measurements of the process variables are needed to develop the control algorithm and adapt the PI controller parameters. Therefore, no additional soft-sensors are needed.

3. The polynomial based algorithm reduced the ISE value by 3 times, while the statistical analysis-based algorithm was reduced by 2.5 times. The developed methods can be easily implemented in most industrial controllers due to their mathematical simplicity and the absence of external measurement equipment requirement.

4. Glucose accumulation in fed-bach cultivation processes can be avoided by measuring the OUR and substrate feeding rate based indicator that is used for real time feeding rate adaptation. The adaptation of the substrate feeding profile reduced the lost amount of the target protein from 37% in the uncontrolled process to 3.8%.

SANTRAUKA

ĮVADAS

Tyrimo aktualumas Per pastaruosius kelis dešimtmečius biotechnologinių procesų taikymo svarba pramonėje smarkiai išaugo [1, 2]. Tai nulėmė pelningumo didėjimas ir produkcijos kokybės gerėjimas bei nauji pramoninių technologijų standartai ar kiti veiksniai. Adaptyvūs valdymo algoritmai daro esminę įtaką technologijų pažangai ir efektyvumui. Per pastaruosius dvidešimt metų buvo įdėta daug pastangų kuriant ir plėtojant novatoriškus adaptyvaus valdymo metodus. Akademinė bendruomenė pasiūlė įvairius adaptyvaus valdymo metodus, tačiau jie paprastai būna sudėtingi, jiems sukurti ir optimizuoti reikia daug laiko ir žinių. Labiausiai žinomi šioje mokslo šakoje intensyviai dirbančių mokslininkų K. Astromo, B. Wittenmarko, M. M. Guptos darbai. Vis dėlto adaptyvaus valdymo algoritmų, veikiančių nežinomoje, netiesinėje ir kintančioje laike aplinkoje, kuri būdinga biotechnologiniams procesams, kūrimo uždaviniai teoriniu ir praktiniu požiūriais dar nėra tinkamai išspręsti, todėl adaptyvūs valdymo algoritmai vis dar retai taikomi biotechnologiniams procesams valdyti netgi pirmaujančiose pasaulio biotechnologijų bendrovėse. Todėl, norint sėkmingai naudoti adaptyvius valdymo algoritmus pramonėje, reikia, kad jie būtų paprasti, lengvai modifikuojami ir implementuojami.

Mokslinė ir technologinė problema bei darbinė hipotezė

Biotechnologiniai procesai yra vieni sudėtingiausių valdymo objektų, turinčių visas valdymą apsunkinančias savybes: netiesinius ryšius tarp proceso kintamųjų, laike besikeičiančias dinamines savybes, jutiklių, galinčių užtikrinti patikimą proceso stebėjimą, trūkumą. Todėl efektyvių valdymo sistemų kūrimas yra aktualus bioinžinerijos uždavinys. Adaptyvaus valdymo algoritmų poreikis yra didelis, jie reikalingi kuriant naujus ir tobulinant biotechnologinius procesus tiek mokslinėse laboratorijose, tiek pramonės įmonėse.

Tyrimo objektas – adaptyvūs valdymo algoritmai, skirti biotechnologiniams procesams.

Tyrimo tikslas – sukurti tipinių biotechnologinių procesų adaptyvaus valdymo algoritmus, tinkamus įdiegti standartiniuose pramoniniuose valdikliuose.

Tyrimo uždaviniai

1. Biotechnologinių procesų tiesioginio ir netiesioginio adaptyvaus valdymo algoritmų kūrimas ir tyrimas.
2. Biotechnologinių procesų adaptyvaus valdymo algoritmų, paremtų neraiškiųjų aibių logika, stiprinimo numatymu ir statistine analize, kūrimas ir tyrimas.

3. Adaptyvaus valdymo algoritmų taikymas pagrindiniams periodiniams ir periodiniams su pamaitinimu proceso parametrams valdyti (santykinis biomasės augimo greitis, ištirpusio deguonies kiekis, pH).
4. Valdymo algoritmų testavimas, derinimas ir eksperimentinis tyrimas valdant periodinius ir periodinius su pamaitinimu fermentacijos procesus.

Mokslinis naujumas

Šioje daktaro disertacijoje pateikiami penki paprasti adaptyvaus valdymo algoritmai biotechnologiniams procesams valdyti: neraiškiųjų aibių logika pagrįstas adaptyvus PI reguliatoriaus parametrų valdymas, stiprinimo numatymu pagrįstas adaptyvus PI reguliatoriaus parametrų valdymas, statistine analize pagrįstas adaptyvus PI reguliatoriaus parametrų valdymas, polinomais pagrįstas adaptyvus PI reguliatoriaus parametrų valdymas ir substrato padavimo profilio adaptyvus valdymas. Neraiškiųjų aibių modeliai dažnai pasižymi dideliu sudėtingų taisyklių ir narystės funkcijų kiekiu. Tokius modelius sudėtinga kurti ir derinti. Pasiūlytas neraiškiųjų aibių modelis susideda iš 4 paprastų taisyklių, kurios sudaromos remiantis procesų operatoriaus patirtimi, ir 6 narysčių funkcijų, taip išlaikant mažą modelio kompleksiškumą. Stiprinimo numatymu paremti modeliai dažnai reikalauja papildomų proceso kintamųjų parametrų matavimo kuriant ir realizuojant reguliatoriaus derinimo parametrų adaptavimą. Sukurti stiprinimo numatymu paremti adaptyvieji valdymo algoritmai remiasi tik reguliatoriaus įėjimo ir išėjimo parametrais, taip nereikalaujant papildomų matavimų reguliatoriaus parametrų adaptacijai realizuoti. Adaptyvių valdymo algoritmų kūrimas taip pat pasižymi sudėtingais veiksmais (išvestinių skaičiavimas, aproksimavimas ir kt.). Pasiūlyti išėjimo signalo statistine analize ar polinomais paremti adaptyvūs valdymo algoritmai remiasi tik bazinėmis matematinėmis operacijomis perskaičiuojant PI reguliatoriaus derinimo parametrus. Substrato padavimo adaptacijos modelis orientuotas į efektyvios substrato padavimo strategijos, užtikrinančios proceso valdomumą ir pakankamą bioproceso produktyvumą, sukūrimą. Sukurtas substrato padavimo adaptacijos modelis yra pagrįstas visiškai eksperimentiniais duomenimis, todėl jį gali nesunkiai pritaikyti bioprocesų operatoriai, dirbantys su realiais pramoniniais procesais ir neturintys specialių žinių matematinio modeliavimo ir valdymo srityse.

Praktinė reikšmė

1. Sukurti sprendimai yra svarbūs nacionaliniu ir tarptautiniu mastu, adaptyviojo valdymo algoritmai gali būti diegiami nacionalinėse biotechnologijų bendrovėse, pavyzdžiui:
   (a) Northway Biotech, UAB
   (b) Celltechna, UAB

2. Šioje daktaro disertacijoje pateikiamiems metodams plėtoti galimybes sudarė Europos Sąjungos struktūrinių fondų finansuojamas projektas „Biotechnologinių procesų adaptyvaus valdymo algoritmų ir sistemų kūrimas ir tyrimai“ (Nr. 01.2.2-LMT-K-718), 2017–2022 m.

3. Vienas iš sukurtų algoritmų buvo patentuotas Lietuvos patentų biure: Galvanauskas Vytautas (išradimo autorius); Simutis Rimvydas (išradimo autorius); Levišauskas Donatas (išradimo autorius); Urniežius Renaldas (išradimo autorius); Vaitkus Vygandas (išradimo autorius); Tekorius Tomas (išradimo autorius); **Butkus Mantas** (išradimo autorius); Survyla Arnas (išradimo autorius). Maitinimo profilių, skirtų valdyti pusiau periodinius rekombinantinių *E. coli* kultivavimo procesus, formavimo ir adaptavimo būdas / išradėjai: V. Galvanauskas, R. Simutis, D. Levišauskas, R. Urniežius, V. Vaitkus, T. Tekorius, **M. Butkus**, A. Survyla; savininkas: Kauno technologijos universitetas. LT 6861 B. 2021-11-10.

Tyrimo rezultatų aprobavimas

Daktaro disertacija remiasi dviem pagrindiniais straipsniais, publikuotais tarptautiniuose moksliniuose žurnaluose, turinčiuose cituojamumo rodiklį „Clarivate Web of Science“ duomenų bazėje. Iš viso rezultatai publikuoti 3 moksliniuose straipsniuose. Vienas iš sukurtų metodų patentuotas Lietuvoje. Sukurti sprendimai buvo pristatyti trijose tarptautiniu mastu pripažintose konferencijose Lietuvoje, Italijoje ir Prancūzijoje.

Atlikti tyrimai buvo teigiamai įvertinti tiek tarptautiniu, tiek šalies mastu.

Ginti teikiami teiginiai

1. Neraiškiąja logika pagrįstam adaptyvaus valdymo algoritmo kūrimui nereikia gilių žinių apie procesą, tam pakanka žinių, kurias galima gauti kokybiškai suprantant įvairių svarbių proceso kintamųjų sąsajas. Tokių žinių paprastai turi proceso operatoriai. Sukurtasis neraiškiąja logika pagrįstas adaptyviojo valdymo algoritmas, kompensuodamas nuokrypius, kuriuos gali sukelti įvairūs proceso trikdžiai ar įrangos gedimai, pranoksta kitus adaptyvius metodus, todėl šis algoritmas tinka biotechnologiniams procesams valdyti.

2. Adaptyvieji valdymo algoritmai, pagrįsti stiprinimo numatymo metodu, kai parametrams adaptuoti naudojami tik reguliatoriaus įėjimo ir išėjimo kintamieji, yra tinkami biotechnologiniams procesams valdyti. Sukurtasis stiprinimo numatymu pagrįstas algoritmas, veikdamas ekstremaliomis darbo sąlygomis, kai reikia sekti užduotąją vertę plačiame diapazone ir kompensuoti trikdžius, lenkia įprastinius PID reguliatorius su pastoviais reguliavimo parametrais. Naudojant

tik reguliatoriaus įvesties ir išvesties kintamuosius, atliekant reguliatoriaus derinimo parametrų adaptavimą, nereikia papildomų parametrų matavimo realiu laiku. Todėl adaptyvus valdymo algoritmas gali būti įdiegtas nereikalaujant papildomų jutiklių papildomiems matavimams atlikti.

3. Sukurti adaptyvaus valdymo algoritmai, pagrįsti grįžtamojo ryšio signalų statistine analize ir polinomais, tinka periodiniams su pamaitinimu biotechnologiniams procesams valdyti. Šie algoritmai gali būti taikomi pH ir ištirpusio deguonies koncentracijai valdyti esant pastoviai nustatytai vertei fermentacijos procesuose. Šie algoritmai gali būti diegiami pramoniniuose valdikliuose, kadangi PI(D) reguliatoriaus parametrai perskaičiuojami pasitelkiant bazinius matematinius veiksmus.

4. Gliukozės sankaupų bioprocesuose galima išvengti matuojant ištirpusio deguonies suvartojimo greičiu ir substratų tiekimo greičiu pagrįstą rodiklį (sunaudoto deguonies kiekio ir per pasirinktą auginimo laiko intervalą į bioreaktorių patiektos gliukozės kiekio santykis), kuris taikomas bakterijų su substrato limitavimu kultivavimo procesams, kultivavimo metu koreguojant substrato tiekimo greitį.

1. BIOTECHNOLOGINIŲ PROCESŲ VALDYMAS

Įvairių šių dienų iššūkių ir problemų neįmanoma išspręsti be biotechnologijų. Medicinos, farmacijos, aplinkosaugos ir daugelis kitų pramonės šakų labai priklauso nuo bioinžinierių sukurtų produktų. Norint pabrėžti biotechnologinių procesų kontrolės poreikius ir svarbą, ypač gaminant didelės vertės ir (arba) didelės apimties produktus, viena iš sričių, į kurią reikia atkreipti dėmesį, yra naujų procesų kūrimas [7]. Jei randama ar genetiškai sukuriama nauja medžiaga, bioinžinierius nustatys tinkamas aplinkos sąlygas tiksliniam produktui augti ir gaminti. Šie veiksniai paprastai apima temperatūros, pH, ištirpusio deguonies koncentracijos, santykinio augimo greičio ir kitų parametrų valdymą. Nors minėtiems kintamiesiems dažnai pateikiamos užduotos vertės, kurios mažesnio masto reaktoriuose gali būti palaikomos PID reguliatoriais, optimalus šių proceso kintamųjų trajektorijų nustatymas vis dar yra sudėtingas inžinerijos uždavinys. Būtent šis optimalus maistinių medžiagų tiekimas dažniausiai nerealizuojamas biologinėje laboratorijoje, tačiau jis suteikia daug galimybių pagerinti gamybą naudojant optimalų valdymą. Nesvarbu, ar gamybai naudojamos bakterijos, pavyzdžiui, *E. coli*, mielės, grybeliai, ar gyvūnų ląstelės, jos sudarytos iš tūkstančių skirtingų junginių, kurie tarpusavyje reaguoja šimtais ar daugiau reakcijų. Visos reakcijos yra griežtai reguliuojamos molekuliniu ir genetiniu pagrindu. Šių procesų matematinis modeliavimas, aprašantis augimą ir gamybą, reikalauja daug konkrečiam procesui būdingų žinių. Be to, labai svarbu modeliu pagrįsti ląstelės ir aplinkos būklės įvertinimą, nes paprastai neįmanoma atlikti ląstelės vidinių procesų ir maistinių medžiagų koncentracijos matavimų realiu laiku.

Be palyginti paprastų fizikinių parametrų, tokių kaip temperatūra, pH, ištirpusio deguonies ar anglies dioksido koncentracija, valdymo, tik keli kintamieji paprastai kontroliuojami atsižvelgiant į nustatytą vertę. Ryškiausias pavyzdys – biomasės augimo greitis, kai siekiama kuo greičiau pasiekti didelę ląstelių koncentraciją reaktoriuje. Jei maistinių medžiagų tiekimas palaikomas virš tam tikro lygio, gaunama neribojančio augimo elgsena, todėl modeliu pagrįstam valdymui galima naudoti nestruktūrizuotus modelius. Tačiau reikia vengti maistinių medžiagų pertekliaus, nes kai kurie organizmai, pavyzdžiui, kepimo mielės, inicijuoja perteklinę medžiagų apykaitą su produktais, kurie vėlesniuose auginimo etapuose gali būti inhibitaciniai. Kai kurių produktų, pavyzdžiui, antibiotiko penicilino, atveju organizmas turi augti lėtai, kad būtų pasiektas didelis gamybos greitis. Šiems vadinamiesiems antriniams metabolitams reikia mažų, bet neišnykstančių kai kurių ribojančių substratų koncentracijų.

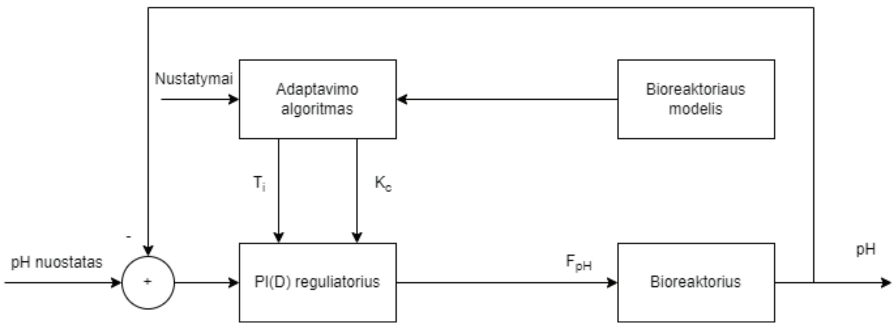
Valdymo sistemos projektavimui didelę įtaką daro proceso netiesiškumų skaičius. Klasikiniai reguliatoriai, tokie kaip proporcinis-integralinis-diferencialinis (PID) arba proporcinis-integralinis (PI), yra tinkami, jei susiduriama su labai nedideliu netiesiškumu. Tačiau, esant dideliam netiesiškumų skaičiui, tokie tiesiniai modeliai yra neveiksmingi, nes net nedideli trikdžiai gali priversti procesą nukrypti nuo darbinio taško [11].

Valdymo kokybei įtakos turi reguliatoriaus gebėjimas užtikrinti stabilų veikimą, veikiant proceso dinamikai ir trikdžiams [12, 13, 14]. Tikslus technologinių parametrų valdymas mikroorganizmų auginimo procesuose yra būtinas, kad būtų išlaikytas norimų technologinių režimų ir procesų atkuriamumas. Tačiau periodinio ir periodinio su pamaitinimu kultivavimo procesų dinaminiai parametrai kultivavimo ciklo metu labai kinta. Todėl įprasti PI(D) reguliatoriai su pastoviais reguliavimo parametrais negali užtikrinti reikiamos valdymo kokybės [15].

1.1. Adaptyvus biotechnologinių procesų valdymas

Adaptyvusis valdymas – tai metodų, kuriais užtikrinamas automatinis reguliatorių reguliavimo parametrų adaptavimas realiuoju laiku, kad būtų pasiektas arba išlaikytas pageidaujamas valdymo sistemos veikimo lygis, kai dinaminiai modelio parametrai nežinomi ir (arba) kinta laikui bėgant, rinkinys [16]. Pirmiausia panagrinėkime atvejį, kai dinaminio modelio parametrai nežinomi, bet yra pastovūs (bent jau tam tikroje veikimo srityje). Tokiais atvejais, nors reguliatoriaus struktūra apskritai nepriklausys nuo konkrečių modelio parametrų verčių, efektyviai sureguliuoti reguliatoriaus parametrų negalima nežinant jų verčių. Adaptyviojo valdymo metodai gali užtikrinti automatinę reguliatoriaus parametrų derinimo procedūrą. Tokiais atvejais adaptacijos poveikis išnyksta laikui bėgant. Tačiau, pasikeitus eksploatavimo sąlygoms, adaptacija gali veikti neefektyviai, tad gali tekti parametrus derinti iš naujo.

Dabar panagrinėkime atvejį, kai įrenginio dinaminio modelio parametrai laike kinta nenuspėjamai. Tokių atvejų pasitaiko arba dėl to, kad keičiasi aplinkos sąlygos, arba dėl to, kad buvo sudaryti supaprastinti tiesiniai netiesinių sistemų modeliai. Tokių situacijų gali pasitaikyti ir dėl to, kad sistemos parametrai lėtai kinta laike. Norint pasiekti ir išlaikyti priimtiną valdymo sistemos veikimo lygį, kai vyksta dideli ir nežinomi modelio parametrų pokyčiai, reikia apsvarstyti adaptyvių reguliatorių naudojimą. Daugiau informacijos apie adaptyviosios valdymo sistemos veikimą galima gauti, jei panagrinėsime 1.1.1 paveiksle pavaizduotą reguliatoriaus projektavimo ir derinimo procedūrą.

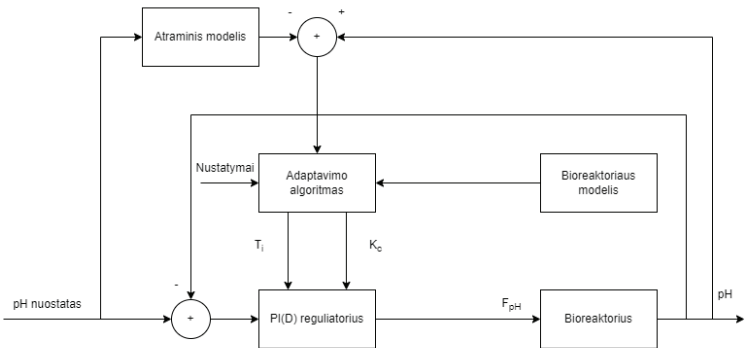


1.1.1 pav. Adaptyvaus pH reguliatoriaus pavyzdys

Norint suprojektuoti ir tinkamai parinkti reguliatoriaus parametrus, reikia sudaryti biotechnologinio proceso dinaminį modelį [17]. Bioreaktoriaus dinaminį modelį galima nustatyti pagal eksperimentinius duomenis. Galima teigti, kad reguliatoriaus projektavimas ir derinimas atliekamas pagal surinktus sistemos duomenis. Adaptyviąją valdymo sistemą galima laikyti pirmiau minėtos projektavimo ir derinimo procedūros įgyvendinimu realiuoju laiku. Reguliatoriaus parametrų derinimas bus atliekamas realiuoju laiku remiantis realiuoju laiku gaunamais sistemos duomenimis.

1.1.1. Tiesioginio adaptyvaus valdymo algoritmai

Valdymo algoritmo našumas gali būti apibrėžtas vertinant dinamines sistemos charakteristikas. Tokiu atveju reguliatorius yra kuriamas taip, kad nagrinėjamam objekto modeliui uždarojo kontūro sistema turi užduotos dinaminės sistemos charakteristikas. Tokiu atveju sukuriamas atraminis modelis, kuriame sistema realizuojama su užduotu norimu našumu. Skirtumas tarp valdomo objekto ir atraminio modelio išėjimų yra lygus nuliui, jei pradinės sąlygos yra vienodos abiem modeliams.

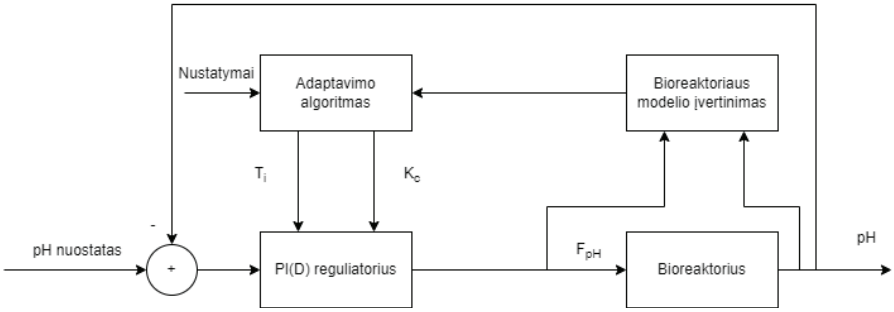


1.1.2 pav. Tiesioginio adaptyvaus valdymo algoritmo struktūrinė schema

Ši schema pagrįsta pastebėjimu, kad skirtumas tarp realaus objekto išėjimo ir atraminio modelio išėjimo yra skirtumo tarp realaus ir pageidaujamo signalo našumo matas. Šią informaciją (kartu su kita informacija) adaptacijos algoritmas naudoja tiesiogiai koreguodamas reguliatoriaus parametrus realiuoju laiku, kad realaus modelio paklaida asimptotiškai artėtų prie nulio.

1.1.2. Netiesioginio adaptyvaus valdymo algoritmai

Netiesioginio adaptyvaus valdymo struktūrinė schema pateikta 1.1.3 paveiksle.



1.1.3 pav. Netiesioginio adaptyvaus valdymo algoritmo struktūrinė schema

Šio metodo pagrindinė idėja yra ta, kad tinkamą reguliatorių galima suprojektuoti realiu laiku, jei biotechnologinio proceso modelis įvertinamas realiu laiku pagal turimus įėjimo ir išėjimo matavimus. Schema vadinama netiesiogine, nes reguliatoriaus parametrai apskaičiuojami dviem etapais:

1. Objekto parametrų įvertinimas realiu laiku.
2. Reguliatoriaus parametrų apskaičiavimas remiantis apskaičiuotais objekto parametrais.

Netiesioginio adaptyvaus valdymo schema siūlo daugybę valdymo dėsnių ir parametrų įvertinimo metodų derinių. Norint geriau suprasti, kaip veikia šios netiesioginio adaptyvaus valdymo schemos, tikslinga išsamiau panagrinėti modelio parametrų įvertinimą realiu laiku [21, 22, 23].

1.2. Esamų adaptyvių biotechnologinių procesų valdymo algoritmų apžvalga

Intensyvi pasaulinė konkurencija, verslo strategijos, kurios daugiausia grindžiamos pelnu, sparčiai besivystančios socialinės ir ekonominės sąlygos, didelis susidomėjimas geresnės kokybės valdymu, didėjantis susirūpinimas sauga ir griežtos aplinkosaugos normos skatina daugelį pramonės šakų automatizuoti savo veiklą naudojant pažangias valdymo sistemas [12, 13, 14]. Sukurti įvairaus sudėtingumo adaptyvaus valdymo algoritmai, skirti biotechnologinių procesų parametrams valdyti kintančiomis laiko sąlygomis. Algoritmas, pagrįstas proceso tendencijų modeliais ir proceso kintamųjų matavimais kultivacijos metu [24, 25], užtikrina kokybišką valdymą ekstremaliomis darbo sąlygomis. Tačiau modeliu pagrįsto valdymo algoritmo kūrimas užima daug laiko, be to, norint atlikti proceso kintamųjų matavimus kultivacijos metu, dažniausiai reikia įdiegti papildomą matavimo įrangą. Ekspertiniai, žiniomis pagrįsti adaptyvieji valdymo algoritmai yra efektyvūs, tačiau jiems sukurti neretai reikia gilių žinių apie biotechnologinį procesą [11, 26]. Adaptyvusis valdymas pagal modelį ir nuorodą (angl. *Model-reference adaptive control,* MRAC) remiasi proceso etaloniniu modeliu, kuris apibrėžia, kaip proceso išvestis turėtų reaguoti į apibrėžtą signalą [55]. Nors MRAC yra gera alternatyva PID, ją reikia derinti kiekvienam konkrečiam procesui, o derinimas priklauso nuo vėlavimo, inertiškumo ir kitų veiksnių. Nežinomiems procesams reguliatorius turi būti derinamas eksperimentiškai, o tai gali būti trūkumas siekiant šiuos metodus įgyvendinti pramoniniuose bioreaktoriuose [11]. Bioreaktoriams adaptyviai valdyti sukurti įvairūs stiprinimo numatymo metodu pagrįsti valdymo algoritmai. Šiuose valdymo algoritmuose deguonies įsisavinimo greitis [62, 63] ir anglies dioksido išsiskyrimo greitis [64, 65] naudojami kaip stiprinimo numatymo kintamieji. Valdymo sistemose šie parametrai įvertinami pagal išmetamųjų dujų analizės, kuri atliekama kultivacijos metu, rezultatus. Norint praktiškai įgyvendinti minėtus algoritmus, būtina, kad bioreaktoriaus sistemoje būtų įrengtas išmetamųjų dujų analizatorius.

1.2.1. Biomasės santykinio augimo greičio valdymas

Vienas iš svarbiausių biotechnologinių procesų valdymo inžinerijos uždavinių – sukurti paprastus ir patikimus metodus, kuriuos būtų galima naudoti pramoninių bioreaktorių santykinio augimo greičiui stebėti ir valdyti. Nepaisant to, šiuo metu daugumoje pramoninio masto bioreaktorių dažniausiai naudojamos gana paprastos valdymo sistemos, nors akademinėje bendruomenėje plačiai diskutuojama apie pažangius sprendimus [58].

Biomasės santykinį augimo greitį, kurį galima apibūdinti kaip biomasės absoliutaus augimo greičio ir kultūroje sukaupto biomasės kiekio santykį, galima laikyti vienu iš svarbiausių biotechnologinių procesų kintamųjų. Jis turi įtakos ne tik kultūros fiziologinei būklei, bet ir lemia produktų kiekį ir kokybę [69, 70, 71]. Tokie poveikiai, kaip substrato limitavimas ar kaupimasis, yra gana paplitę periodiniuose su pamaitinimu procesuose. Tačiau su šiais poveikiais galima susidoroti, jei jie tinkamai kontroliuojami. Gerai parinktas valdymo algoritmas gali užtikrinti didelę produkto koncentraciją ir didelį ląstelių tankį. Tai galima pasiekti palaikant tam tikrą substrato koncentraciją, todėl kontroliuojamas biomasės augimo greitis [12], kurį galima valdyti keičiant substrato padavimo greitį. Daugeliu atvejų biotechnologiniai procesai valdomi naudojant PID (proporcinius-integralinius-diferencialinius) reguliatorius, kurie paprastai veikia pramoninėse valdymo sistemose. Šiose sistemose paprastai kontroliuojama temperatūra, pH ir kiti proceso kintamieji. Valdymo kokybė labai priklauso nuo reguliatoriaus ir jo derinimo bei jo gebėjimo susidoroti su proceso kintamumu ir trikdžiais [12, 14, 13]. Įprasti PID reguliatoriai, kuriuose naudojami pastovūs derinimo parametrai, negali pasiekti reikiamo proceso valdymo tikslumo, nes dinamika darbo metu labai kinta. Akademinė bendruomenė pasiūlė įvairių PID reguliatoriaus parametrų adaptavimo metodų, kuriais atsižvelgiama į kintančias laike darbo sąlygas: taisyklėmis grindžiamas neraiškiųjų aibių sistemas [26], stiprinimo numatymo metodus [58, 89] ir kitus metodus [59, 60, 61, 12, 13, 74, 75]. Pasiūlyti metodai suteikia teorinį ir praktinį pagrindą, leidžiantį panaudoti adaptyviojo valdymo algoritmus valdant biotechnologinius procesus, ir pabrėžia, kad šių valdymo metodų taikymas gali reikšmingai padidinti valdymo sistemų efektyvumą.

1.2.2. Ištirpusio deguonies koncentracijos valdymas

Ištirpusio deguonies koncentracijos ir pH valdymo sistemų kūrimo metodai, pagrįsti dirbtinių neuroninių tinklų modeliais, pateikti [82, 83]. A. Mészáros ir kt. pristato dirbtinį neuroninį tinklą, kuris yra apmokytas prognozuoti valdomų procesų netiesinę dinamiką, o atvirkštiniai neuroniniai tinklai yra naudojami valdymo sistemose kaip grįžtamojo ryšio reguliatoriai [82]. Du, Xianjun ir kt. sukūrė radialinėmis bazinėmis funkcijomis pagrįstą neuroninio tinklo adaptyvųjį PID reguliatorių ištirpusio deguonies koncentracijai valdyti [83]. Tokiam neuroninų tinklų modeliu pagrįstų valdymo sistemų kūrimui reikia pakankamo kiekio informatyvių proceso duomenų ir laiko sąnaudų modeliui apmokyti. Dėl šių priežasčių sudėtingų valdymo sistemų taikymas pramoninių bioprocesų valdymo inžinerijos praktikoje nėra patrauklus. Taip pat sukurtos ištirpusio deguonies koncentracijos valdymo sistemos [88, 84], kuriose PID (PI) reguliatoriaus adaptacijai nereikia papildomų proceso kintamųjų matavimų. Reguliatoriaus parametrų adaptavimas grindžiamas statistine reguliatoriaus įvesties ir išvesties duomenų analize kultivacijos metu. Tačiau optimalios algoritmų derinimo parametrų vertės nustatomos taikant „bandymų ir klaidų“ metodą, kuris reikalauja daug laiko. Įvairūs kiti PID reguliatoriaus parametrų adaptavimo metodai pateikti [74, 75, 77].

1.2.3. pH valdymas

Vienas iš dažnai biotechnologiniuose procesuose valdomų parametrų yra terpės pH lygis. pH lygio reguliavimas padeda užtikrinti produkto kokybę ir sumažina kontroliuojamo objekto įrangos koroziją. Pakankamai veiksminga pH kontrolė taupo pH lygiui kontroliuoti naudojamą reagentą. pH lygio kontrolė naudojama įvairiose srityse:

1. Auginant mikroorganizmus [90].
2. Druskų kristalizavimo procesuose chemijos pramonėje [91].
3. Paviršių apdorojimo procesuose [92].
4. Fermentacijos procesuose [93].
5. Farmacijoje [94].
6. Vandens minkštinimo, valymo ir kituose procesuose [95, 96].

Kokybišką pH kontrolę sunku užtikrinti dėl daugelio priežasčių: labai didelio biocheminių procesų, titravimo kreivių ir paties pH matavimo netiesiškumo, didelio mikroorganizmų jautrumo net mažiems laikiniems pH lygio nuokrypiams auginimo terpėje ir pH jutiklių dreifo [97, 98].

Akademinė bendruomenė pasiūlė įvairių PID reguliatoriaus parametrų adaptavimo metodų kokybiškam pH valdymui. Vis dėlto dauguma jų turi trūkumų [100, 105], pavyzdžiui, sudėtingas reguliatoriaus projektavimas, didžiulės laiko investicijos kūrimui, brangi aparatinė įranga arba daug derinimo parametrų. Todėl labai svarbu parengti paprastus, patikimus ir lengvai įgyvendinamus tikslaus pH valdymo metodus.

2. SUKURTI ADAPTYVŪS BIOTECHNOLOGINIŲ PROCESŲ VALDYMO ALGORITMAI

Šiame darbe naudojami jau sukurti ir identifikuoti matematiniai biotechnologinių procesų modeliai, kurie pateikiami fundamentaliomis matematinėmis išraiškomis. Šiuose modeliuose nefigūruoja juodosios dėžės elementai, todėl modeliams nereikia tirti parametrų neapibrėžtumų įtakos. Jie naudojami tiriant valdymo algoritmus, atliekant imitacinį modeliavimą.

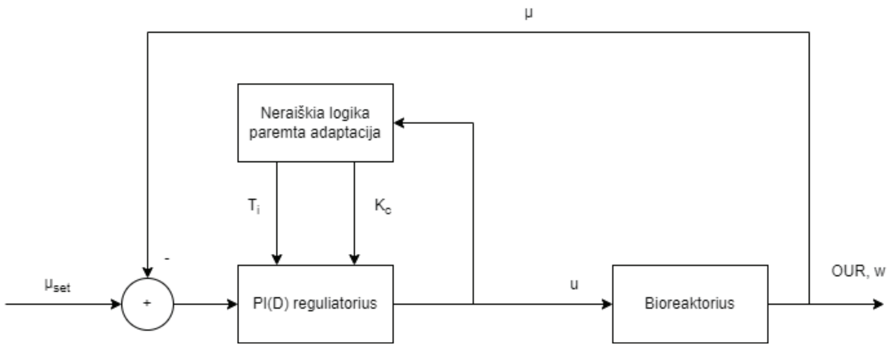
2.1. Neraiškiųjų aibių logika pagrįstas adaptyvus santykinio augimo greičio valdymas

2.1.1. Biotechnologinio proceso matematinio modelio sudarymas

Šiame tyrime biotechnologinis procesas buvo modeliuojamas naudojant *Escherichia coli BL21* matematinį modelį MATLAB aplinkoje. Ši ląstelė taip pat turėjo pBR322 plazmidės darinį ir buvo auginama rekombinantinio maitinimo proceso metu. Šis rekombinantinis baltymas dažnai naudojamas tipiniuose bioreaktorių masto procesuose. Pasirinkto rekombinantinio baltymo kultivavimą galima apibūdinti kaip dviejų fazių procesą. Pirmojo etapo tikslas – bioreaktoriuje sukaupti biomasę. Santykinis augimo greitis šioje fazėje paprastai yra santykinai didelis. Antrojo etapo metu gaminamas rekombinantinis baltymas. Aprašytų dviejų fazių metu kiekvienai fazei buvo nustatyta ir palaikoma skirtinga optimali temperatūra. Modeliuojamame procese pirmojo etapo metu buvo palaikoma 37 °C temperatūra, taip maksimaliai padidinant biomasės augimą iki optimalaus lygio. Gamybos proceso pradžioje temperatūra buvo sumažinta iki 32 °C. Substrato tiekimo greičio profiliai ir indukcijos laikas taip pat turi didelę įtaką proceso našumui. Todėl jie taip pat optimizuojami pagal modelį. Indukcijos laikas (8 val.) buvo nustatytas [106] taikant modeliais pagrįstus optimizavimo metodus. Atliekant modeliu pagrįstą proceso optimizavimą [106], buvo naudojamas proceso našumo (produktyvumo) rodiklis, lygus bendram siektinam baltymų kiekiui proceso pabaigoje. Tiriamame procese biomasės ir tikslinių baltymų analizei taikomi realaus laiko matavimo metodai išsamiai aprašyti [106]. Pagrindiniai proceso kintamieji šiuo atveju buvo modeliuojami programiniais jutikliais. Aprašytam biotechnologiniam procesui modeliuoti buvo naudojamas [106] pateiktas matematinis modelis. Remiantis kitais tyrimais, biomasės augimui ir baltymų gamybai kontroliuoti naudojami iš anksto optimizuoti substrato tiekimo profiliai [106]. Tokio tipo atviro kontūro sistema gali būti efektyviai valdoma tik tada, kai nėra didelių proceso sąlygų nuokrypių ar įrangos sutrikimų ir (arba) gedimų. To nepadarius, gali atsirasti santykinio augimo greičio nuokrypių, kurie sumažintų proceso našumą. Todėl santykinis augimo greitis buvo pasirinktas kaip pagrindinis kontroliuojamas šio tyrimo kintamasis, kuris būtų palaikomas kontroliuojant substrato padavimą. Šiame tyrime optimaliai santykinio augimo greičio trajektorijai palaikyti buvo naudojamas adaptyvus valdymo algoritmas.

2.1.2. Adaptyvaus valdymo algoritmo kūrimas

Siekiant realizuoti adaptyvų santykinio augimo greičio valdymo algoritmą, buvo sukurtas ir ištirtas PI reguliatorius su neraiškiųjų aibių logika pagrįstu reguliatoriaus parametrų adaptavimu. Įprasto PID reguliatoriaus taikymas yra ribotas dėl nestacionaraus ir netiesinio aprašytų biotechnologinių procesų elgesio. Todėl buvo suprojektuotas ir įgyvendintas neraiškusis modelis, skirtas PI reguliatoriaus parametrams adaptuoti kultivacijos metu. Kadangi santykinis augimo greitis negali būti matuojamas tiesiogiai, signalui apskaičiuoti buvo naudojamas būsenos įvertis ir pagalbinis kintamasis [58]. Šie kintamieji vėliau naudojami grįžtamajame ryšyje. Šiame tyrime nuspręsta, kad deguonies suvartojimo greitis būtų tinkamas papildomas kintamasis dėl to, kad jis ne tik atspindi kultūrų fiziologinę būklę, bet ir turi gerą koreliaciją su santykiniu augimo greičiu. Gauti rezultatai rodo, kad biomasės koncentracijos, apskaičiuotos pagal nustatytą santykinį augimo greitį, gerai atitinka išmatuotas (etalonines) biomasės koncentracijas. 2.1.1 pav. pavaizduota bendra sukurto reguliatoriaus struktūra.



2.1.1 pav. Neraiškiųjų aibių logika paremto valdymo algoritmo struktūrinė schema

Norint sukurti neraiškiųjų aibių logikos pagrindu veikiantį adaptyvųjį PI reguliatorių, reikia apibrėžti sistemos įvesties ir išvesties kintamuosius. Kadangi šio modelio paskirtis yra adaptuoti PI reguliatoriaus parametrus, kaip išėjimo kintamieji buvo pasirinkti reguliatoriaus stiprinimo K_c ir integravimo laiko konstantos T_i parametrai. Tiek T_i , tiek K_c priklauso nuo proceso kintamųjų, kaip aprašyta [58]:

$$K_c \propto k_0 w, \tag{2.1}$$

$$T_i \propto \frac{k_1}{OUR/w + k_2}, \tag{2.2}$$

čia k_0, k_1 ir k_2 yra pastovūs parametrai, kurių reikšmės buvo apskaičiuotos bei išsamiai aptartos [58]. Optimizavimo tikslas – rasti parametrų rinkinį, kuris minimizuotų suprojektuoto valdymo algoritmo sekimo paklaidą (pvz., integralinę laiko absoliutinę paklaidą), kai reguliatoriaus parametrams pritaikyti naudojamos prieš tai paminėtos lygtys. Kadangi PI reguliatoriaus parametrai daugiausia priklauso nuo deguonies suvartojimo greičio OUR ir kultūros svorio w, abu proceso kintamieji turi būti naudojami valdymo algoritme. Siekiant sumažinti algoritmo sudėtingumą, šių dviejų proceso kintamųjų santykis buvo naudojamas kaip algoritmo įvestis, o neraiškiųjų aibių sistemoje panaudota klasikinė versija. Remiantis turimomis euristinėmis žiniomis, buvo sukurtos šios taisyklės, skirtos nagrinėjamam algoritmui:

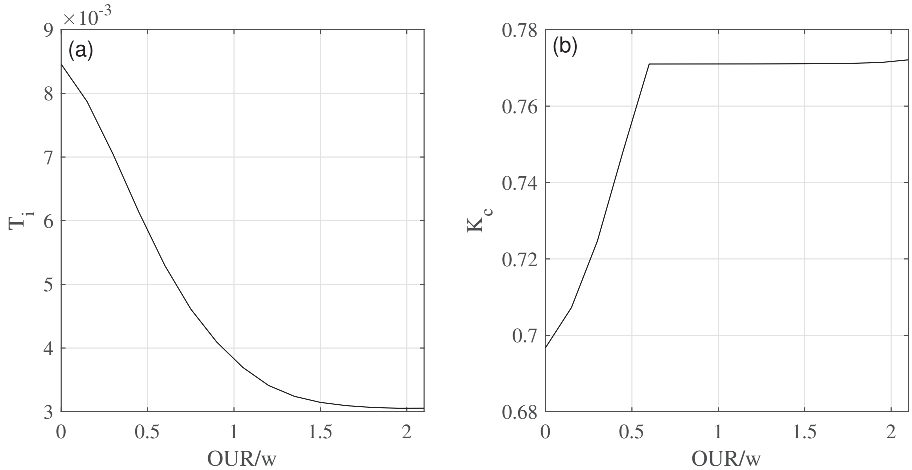
Jei OUR/w santykis yra mažas, tada T_i yra didelė;

Jei OUR/w santykis yra didelis, tada T_i yra maža;

Jei OUR/w santykis yra mažas, tada K_c yra mažas;

Jei OUR/w santykis yra didelis, tada K_c yra didelis.

Tiek neraiškiojo modelio įėjimui, tiek išėjimui buvo pasirinktos dvi Gauso narystės funkcijos. Siekiant išvengti didelio narystės funkcijų persidengimo ir sumažinti modelio kompleksiškumą, kiekvienam parametrui naudojamų narystės funkcijų skaičius buvo sumažintas iki dviejų. Narysčių funkcijų parametrai buvo nustatyti panaudojant genetinį algoritmą. K_c ir T_i priklausomybės pateiktos 2.1.2 pav.



2.1.2 pav. PI reguliatoriaus parametrų kitimas *fuzzy* modelyje: integralinė laiko pastovioji T_i(a), stiprinimas K_c (b)

Genetinio algoritmo vertės buvo perskaičiuotos 20 kartų, siekiant užtikrinti parametrų patikimumą.

2.2. Ištirpusio deguonies koncentracijos valdymas panaudojant stiprinimo numatymą

Ištirpusio deguonies koncentracijos (DOC) dinamika kultūros terpėje gali būti išreikšta paprasta diferencialine lygtimi, pagrįsta DOC masės balansu:

$$\frac{dc}{dt} = K_L a(c_{sat} - c) - OUR, \tag{2.3}$$

čia $K_L a$ deguonies perdavimo koeficientas, s^{-1}, c yra ištirpusio deguonies koncentracija, $\%$, c_{sat} – šios vertės įsisotinimo koeficientas, $\%$, OUR yra deguonies suvartojimo greitis, $mmols^{-1}$.

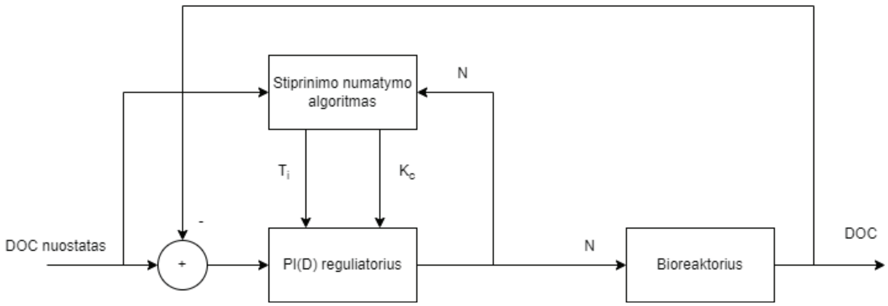
Atlikus šios lygties tiesinimą taško t_k aplinkoje, pridėjus vėlavimą bei įvertinus, kad reguliatoriaus stiprinimo koeficientas K_c yra proporcingas $T_{pr}/K_{pr}/\tau$, o laiko pastovioji T_i yra proporcinga T_{pr}, gaunama 2.4 priklausomybė. Atsižvelgiant į funkcines priklausomybes ir darant prielaidą, kad kultivavimo proceso metu kontroliuojama ištirpusio deguonies koncentracijos vertė yra artima nustatytosios vertės vertei, galima įvertinti reguliatoriaus derinimo parametrų ir reguliatoriaus išėjimo bei nuostato signalo priklausomybių pobūdį:

$$K_c(t_k) = \frac{K_{Kc}}{u(t_k)(c_{sat} - c_{set}(t_k))}, \tag{2.4}$$

$$T_{pr} = \frac{K_{Ti}}{(u(t_k))^2}, \tag{2.5}$$

$$K_{Ti} = k_{Ti}\frac{1}{\alpha q^{\gamma}}, \tag{2.6}$$

čia k_{Ti} ir K_{Kc} yra koeficientai, skirti reguliatoriui derinti, kad būtų pasiektas pageidaujamas valdymo sistemos veikimas (apytikslę koeficiento vertę galima paimti iš pageidaujamų reguliatoriaus derinimo taisyklių). u yra maišymo greitis (valdymo kintamasis), s^{-1}, q oro tiekimo greitis, ls^{-1}, α ir γ yra parametrai, -. Biotechnologiniam procesui imituoti buvo naudojamas *E.coli* maitinimo proceso matematinis modelis, pateiktas [25]. Sukurtos valdymo sistemos struktūrinė schema pateikiama 2.2.1 paveiksle.



2.2.1 pav. Adaptyvaus ištirpusio deguonies koncentracijos valdymo struktūrinė schema

Kaip parodyta 2.2.1 pav., ištirpusios deguonies koncentracijos adaptyvusis valdymo algoritmas reguliatoriaus parametrams apskaičiuoti naudoja tik reguliatoriaus įvesties ir (arba) išvesties signalus, taip išreiškiant sistemos unikalumą. Ištirpusios deguonies koncentracijos matavimai buvo modeliuojami pridedant Gauso triukšmą:

$$c_{elm}(t_k) = c_{el}(t_k) + \sigma Randn, \tag{2.7}$$

čia c_{elm} – išmatuota DOC vertė; σ – standartinis nuokrypis, apskaičiuotas pagal realius matavimus ($\sigma \cong$ 0.2%), $Randn$ – normalizuotų Gauso atsitiktinių skaičių seka. Modeliuojant stiprinimo numatymo ir PI valdymo algoritmus, visų modeliavimo eksperimentų metu buvo naudojamas laiko diskretizacijos žingsnis Δt = 0,18 s.

2.3. pH valdymas panaudojant stiprinimo numatymą

Periodiniuose su pamaitinimu kultivavimo procesuose vandenilio jonų koncentracija gali būti modeliuojama atsižvelgiant į mikroorganizmų augimo, rūgščių ir šarmų tirpalų dozavimo įtaką kontroliuojant pH:

$$\frac{dC_{H^+}}{dt} = (\alpha_1 \mu x + \alpha_2 x) + \frac{F_{pH}(C^0_{H^+} - C_{H^+})}{V} - \frac{F_s C_{H^+}}{V}, \tag{2.8}$$

čia $C^0_{H^+}$ ir C_{H^+} yra vandenilio jonų koncentracijos atitinkamai bioreaktoriuje ir šarminiame tirpale. Tikrosios ir apskaičiuotos $C^0_{H^+}$ vertės gali skirtis, tad turi būti identifikuotos. x – biomasės konsentracija terpėje, g/l, μ – biomasės santykinis augimo greitis, 1/h, F_{pH} – šarminio tirpalo srautas (valdymo kintamasis), l/h, F_s – pamaitinimo srautas, l/h, V – kultivavimo terpės tūris, l, α_1, α_2 – modelio parametrai, kurie turi būti identifikuoti iš eksperimentinių duomenų. Pradinė $C_{H^+}(0)$ vertė yra lygi 10^{-7} mol/l, kas prilygsta pH 7. Šios lygties ištiesinimas taško t_k aplinkoje bei įvertinus, jog reguliatoriaus stiprinimo koeficientas K_c yra proporcingas $T_{pr}/K_{pr}/\tau$, o laiko pastovioji T_i yra proporcinga T_{pr}, gaunamos funkcinės priklausomybės, darant prielaidą, kad auginimo proceso metu kontroliuojama pH vertė yra artima nustatytosios vertės vertei.

Tokiu atveju reguliatoriaus parametrų priklausomybes galima išreikšti lygtimis:

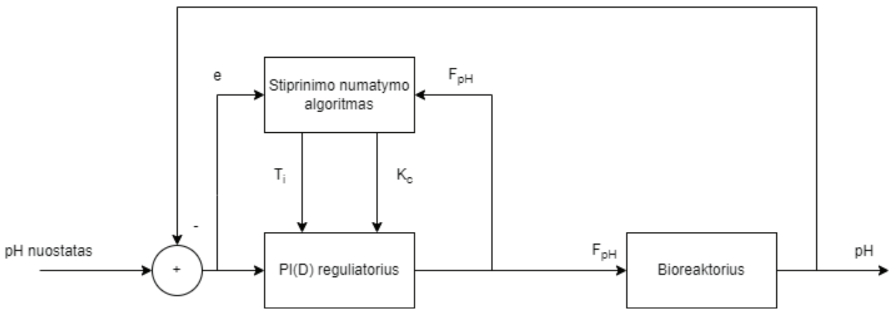
$$K_c(t_k) = \frac{K_{Kc}V}{C^0_{H^+}(t_k) - C_{H^+}(t_k)}, \tag{2.9}$$

$$T_i(t_k) = \frac{k_{Ti}V(t_k)}{F_{pH}(t_k) + F_s(t_k)}, \tag{2.10}$$

čia K_{Kc} ir k_{Ti} yra koeficientas, skirtas reguliatoriui derinti, kad būtų pasiektas pageidaujamas valdymo sistemos veikimas. Remiantis turimais realaus laiko matavimais, galima sukurti šiuos valdymo algoritmus:

1. Valdymo algoritmą, kuriame F_{pH}, F_s, ir V matuojami kultivacijos metu.
2. Valdymo algoritmą, kuriame F_{pH} ir F_s matuojami kultivacijos metu, o naudojama pastovi vidutinė V vertė.
3. Valdymo algoritmą, kuriame F_{pH} ir V matuojami kultivacijos metu, o F_s apskaičiuojamas kaip $F_s = kF_{pH}$.
4. Valdymo algoritmą, kuriame kultivacijos metu matuojamas tik F_{pH}, V laikomas pastoviu ir lygiu vidutinei vertei, o F_s apskaičiuojamas kaip $F_s = kF_{pH}$, čia k yra derinimo parametras.

Ištirto adaptyvaus pH valdymo algoritmo, įgyvendinančio PI reguliatoriaus parametrų adaptaciją su reguliatoriaus išėjimo ir įėjimo signalais kaip stiprinimo numatymo kintamaisiais, schema pavaizduota 2.3.1 pav.



2.3.1 pav. Adaptyvios pH valdymo sistemos struktūrinė schema

Vėlgi nagrinėjamame algoritme PI reguliatoriaus parametrai yra adaptuojami panaudojant tik reguliatoriaus įėjimo ir išėjimo parametrus.

2.4. PI reguliatoriaus parametrų adaptavimas remiantis grįžtamojo ryšio signalų statistine analize

Ankstesni bioreaktorių pH valdymo sistemų tyrimai parodė, kad dėl proceso dinamikos pokyčių būtų tikslinga adaptuoti PI reguliatoriaus parametrus, ypač integralinę laiko konstantą T_i, kuri, kaip paaiškėjo, yra pagrindinis derinimo parametras, priklausantis nuo proceso apkrovos. Optimali valdymo parametro K_c vertė priklauso tik nuo kultūros tūrio, kuris proceso metu šiek tiek kinta [103]. Prieš tai aptartas valdymo algoritmas reikalauja gilių matematikos žinių, todėl nuspręsta pasiūlyti paprastesnę alternatyvą, kur reguliatoriaus parametro T_i derinimas grindžiamas statistine sistemos grįžtamojo ryšio signalo analize. Reguliatoriaus parametrų adaptavimo algoritme grįžtamojo ryšio signalo c_{ave} paklaidos vidutinė vertė apskaičiuojama kultivacijos metu iš slenkančio lango:

$$c_{ave}(k) = \frac{1}{n} \sum_{i=k-n}^{k-1} c_{el}(i), \tag{2.11}$$

$$O_{ffset}(k) = c_{set} - c_{ave}(k), \tag{2.12}$$

čia n yra slenkančio lango ilgis, s, c_{el} – grįžtamojo ryšio signalo vertė, -, o c_{set} yra užduota vertė, -. Be to, absoliuti vidutinė nuokrypio vertė apskaičiuojama iš grįžtamojo ryšio signalo:

$$D_{absave}(k) = \frac{1}{n} \sum_{i=k-n}^{k-1} |c_{el}(i) - c_{ave}(k)|. \tag{2.13}$$

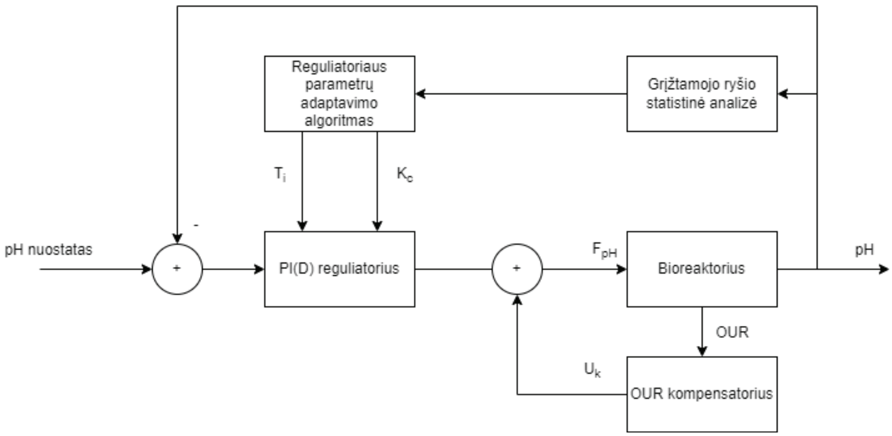
Pirmiau nurodyti statistiniai parametrai taikomi reguliatoriaus integravimo konstantai T_i adaptuoti realaus laiko režimu pagal šią taisyklę:

Jei $|O_{ffset}(k)| > O_{max}$ arba $D_{absave}(k) > D_{max}$,

tada $T_i(k) = T_i(k-1)(1 - a_1 O_{ffset}(k))$.

Kitu atveju $T_i(k) = T_i(k-1)$,

čia a_1, O_{max} ir D_{max} yra derinimo parametrai. Reguliatoriaus stiprinimas K_c šiuo atveju nebuvo keičiamas ir valdomo proceso metu išliko pastovus. Adaptyvusis valdymo algoritmas buvo išbandytas pH valdyti. F_{pH} buvo pasirinktas kaip valdymo kintamasis. Šios sistemos struktūrinė schema pateikta 2.4.1 paveiksle.



2.4.1 pav. Adaptyvaus pH valdymo algoritmo struktūrinė schema

Kad būtų atsižvelgta į bioproceso būsenos kitimą, adaptyvusis PI reguliatorius papildomai palaikomas kompensatoriaus bloko [122], kuris sukuria valdymo poveikį, pagrįstą ištirpusio deguonies greičio įvertinimu kultivacijos metu. Pagrindinius bioproceso būsenos pokyčius gali atspindėti ištirpusio deguonies greitis, kuris taip pat gerai koreliuoja su sistemos dinamika. Šis signalas gali būti naudojamas kaip pasiūlyto hibridinio adaptyviojo valdymo pagrindas. Kompensatoriaus dalis apibrėžia pagrindinę valdymo signalo dalį, o PI išėjimo signalas naudojamas nustatytai pH vertei tiksliau sekti. Vien tik valdymo algoritmo kompensatoriaus dalis negali užtikrinti pakankamai tikslaus pH reikšmės sekimo realiomis sąlygomis dėl kultūroje vykstančių nenu-spėjamų medžiagų apykaitos pokyčių. Be to, kompensatoriaus dalis yra neveiksminga, nes negali kompensuoti didelių trikdžių, sukeliamų pereinamųjų procesų, net jei naudojama su standartiniu PI algoritmu. Yra įvairių galimų technologinių ar specifinių su augimo kontrole susijusių priežasčių, dėl kurių pH reikšmė gali kisti arba ja gali būti manipuliuojama, ir viena iš jų gali būti sistemos veikimo sutrikimai. Reikėtų atsižvelgti į tai, kad manipuliuojant pH užduotąja verte valdomo proceso metu iškraipomi judančio lango duomenys, taigi ir statistinių parametrų įverčiai. Kadangi šios reikšmės naudojamos reguliatoriaus parametrams adaptuoti, grįžtamojo ryšio signalų statistiniais parametrais pagrįstas adaptyvusis valdymo algoritmas yra tinkamesnis pH valdyti tik esant pastoviai užduotai vertei.

2.5. Ištirpusio deguonies koncentracijos valdymas netipiniuose procesuose

Pagrindinis fermentacijos tikslas yra gauti didžiausią įmanomą produkto kiekį per apibrėžtą laiką ir tūrį. Didelis ląstelių tankis tokiu atveju yra būtina didelio produktyvumo sąlyga [123]. Modeliuojant netipinius augimo procesus, kur ištirpusio deguonies kiekio dinamika kinta plačiame diapazone, daromos šios prielaidos:

1. Netipiniuose procesuose deguonies suvartojimo greitis yra tris kartus didesnis, palyginti su įprastu augimo procesu.

2. Į kultivavimo terpę reguliariai įpilama putojimą mažinančios medžiagos, kuri 30 s labai sumažina deguonies suvartojimo greitį (parametras α per šį laiką sumažėja 70 procentų).

Šiuose netipiniuose auginimo procesuose gali atsirasti itin didelių ištirpusio deguonies koncentracijos trikdžių. Paprastai trikdžiai atsiranda tuo metu, kai į auginimo terpę įpilama putojimą mažinančio tirpalo, kad būtų išvengta intensyvaus putojimo, kuris gadina svarbius bioreaktoriaus prietaisus ir kenkia auginimo procesui. Siekiant to išvengti, paprastai atliekamos putų šalinimo procedūros. Šis veiksmas labai sumažina deguonies pernešimo bioreaktoriuje pajėgumą. Kadangi trikdžiai yra tiesiogiai susiję su deguonies suvartojimo greičiu, nuspręsta šį parametrą naudoti adaptuojant PI reguliatoriaus parametrus. Tam naudotas antro laipsnio polinomas:

$$K_p = a_1 + a_2 OUR + a_3 OUR^2, \tag{2.14}$$

$$T_i = b_1 + b_2 OUR + b_3 OUR^2. \tag{2.15}$$

Optimalūs polinomo parametrai buvo nustatyti naudojant genetinį algoritmą. Parametrų nustatymas buvo vykdomas 20 kartų, siekiant patvirtinti jų patikimumą. Genetinio algoritmo parametrai pateikiami 2.5.1 lentelėje.

2.5.1 lentelė. Genetinio algoritmo parametrai

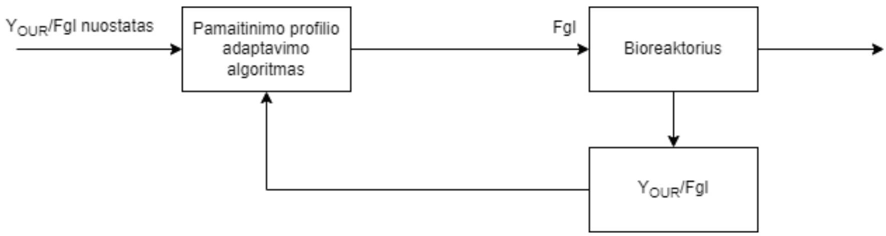
Generacijų skaičius	Individų kiekis vienoje gen.	Mutacijos tikimybė	Kryžminimosi tikimybė
130	300	0,1	0,9

Optimizavimas buvo atliktas minimizuojant integralinės kvadratinės paklaidos vertę. Adaptyvus valdymo algoritmas sumažino ISE vertę beveik tris kartus, palyginti su standartiniu reguliatoriumi, kurio derinimo parametrai pastovūs (PI ISE = 18198, adaptyvaus PI ISE = 6468).

2.6. Maitinimo profilių, skirtų pusiau periodiniams rekombinantinių *E. coli* kultivavimo procesams valdyti, formavimas ir adaptavimas, panaudojant OUR ir substrato tiekimo srautu pagrįstą rodiklį

Kaip teigiama [124], kuriant veiksmingas substratų tiekimo strategijas *E.coli* kultivavimo procesams, svarbiausia yra nustatyti etaloninius substratų tiekimo profilius auginimo ciklams. Tam pirmajame projektavimo procedūros etape, naudojant neribojančio augimo kultivavimo eksperimentų duomenis, turi būti nustatytas gliukozės suvartojimo greičio profilis biomasės augimo ir tikslinės baltymų gamybos fazėje.

Vėliau reikia suprojektuoti įvairius gliukozės tiekimo profilių scenarijus, nustatant skirtingus gliukozės suvartojimo greičio apribojimo lygius kultivavimo proceso metu. Tada, remiantis šių etapų eksperimentiniais duomenimis, reikia parinkti patikimą ir veiksmingą etaloninį gliukozės pamaitinimo profilį, kurį rekomenduojama taikyti pakartotiniuose gamybos procesuose. Etaloninis pamaitinimo profilis, sukurtas taikant siūlomą gliukozės ribojimo metodą, geba kompensuoti trumpalaikius trikdžius, taip nedarant įtakos kultivavimo proceso kokybei. Esant didesniems trikdžiams, kultivavimo procesas gali tapti nestabilus ir neefektyvus dėl bioreaktoriuje susikaupusio didelio gliukozės kiekio. Deja, gliukozės koncentracijos matavimas realiu laiku laboratoriniuose įrenginiuose ir pramoniniuose bioreaktoriuose tebėra neišspręsta problema ir šie matavimai paprastai negali būti naudojami pramoniniams kultivavimo procesams stebėti. Tokiais atvejais reikia netiesiogiai stebėti gliukozės kaupimąsi kultivavimo terpėje ir kultivacijos metu keisti etaloninį pamaitinimo profilį. 2.6.1 pav. parodyta siūloma substrato tiekimo valdymo sistemos struktūra.



2.6.1 pav. Substrato pamaitinimo valdymo sistemos struktūrinė schema

Ji pagrįsta deguonies suvartojimo greičio įvertinimu, kai *E.coli* kultivavimo proceso metu yra maitinama gliukoze. Įvertinimas atliekamas taikant judančio lango metodą, integruojant deguonies suvartojimo greitį ir gliukozės padavimo greitį per šį laikotarpį ir nustatant išeigą $Y_{OUR/Fgl}$. Ši išeiga taip pat nustatoma etaloniniam auginimo procesui. Gauti išeigos rodikliai priklauso nuo *E.coli* kultivavimo fazės ir kinta kultivavimo proceso metu. Jei *E.coli* dėl didelių sutrikimų gliukozės visiškai nesuvartoja, deguonies pasisavinimo išeiga iš paduodamos gliukozės pradeda mažėti.

Proceso reguliatorius lygina tikrąją išeigą su etalonine ir, naudodamas PI algoritmo formą, keičia etaloninį profilį:

$$E(i) = Y_{OUR/Fgl_{etaloninis}}(i) - Y_{OUR/Fgl(i)}(i), \tag{2.16}$$

$$U(i) = U(i-1) + K_c[1 + \frac{dt}{T_i}E(i) - E(i-1)], \tag{2.17}$$

$$Fgl(i) = Fgl_{etaloninis}(i) - U(i), \tag{2.18}$$

$$dt = t(i) - t(i-1), \tag{2.19}$$

čia $E(i)$ yra skirtumas tarp etaloninio ir tikrojo įverčio, $U(i)$ – reguliatoriaus išėjimas, K_c, T_i – reguliatoriaus parametrai, dt – diskretizavimo žingsnis.

Svarbu pažymėti, kad šiuolaikiniuose laboratoriniuose ir pramoniniuose bioreaktoriuose gali būti įrengti prietaisai, kuriais galima matuoti aeracijos dujų sudėtį įėjime ir išėjime bei molinį srautą. Taigi deguonies įsisavinimo greitį galima apskaičiuoti pagal gerai žinomą paprastą lygtį. Ši aplinkybė suteikia gerą galimybę taikyti siūlomą maitinimo profilių pritaikymo algoritmą pramoniniuose bioreaktoriuose.

Valdymo metodo efektyvumas įvertintas remiantis trimis eksperimentais, atliktais Kauno technologijos universiteto laboratorijoje. Profilių tikslumui įvertinti buvo pasirinktos vidutinės absoliutinės paklaidos (MAE) ir vidutinės kvadratinės paklaidos (RMSE) vertės [126].

3. SUKURTŲ METODŲ EFEKTYVUMO VERTINIMAS

3.1. Užduotų verčių apžvalga

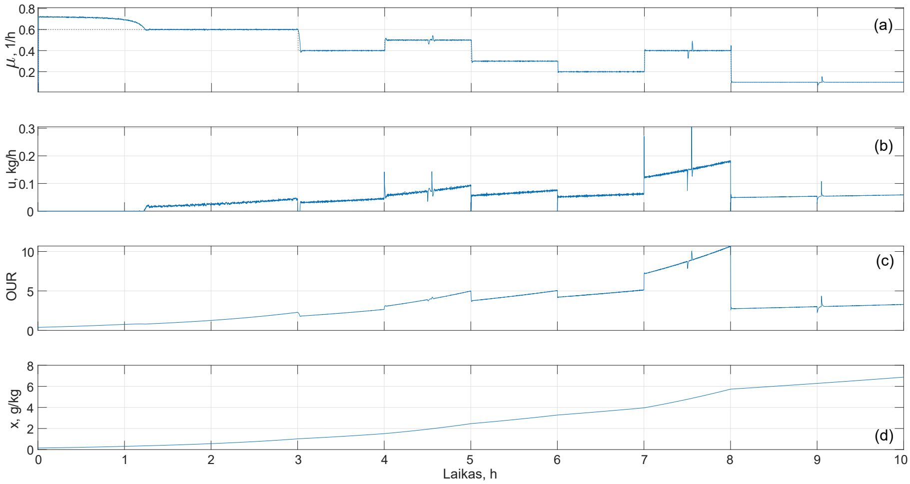
Siekiant įvertinti suprojektuoto valdymo algoritmo elgseną, buvo pasirinkti ir sumodeliuoti tipiniai skirtingų ilgių ir amplitudžių užduotųjų verčių modeliai, įskaitant matuojamo kintamojo trikdžius:

1. Santykinis augimo greitis buvo modeliuojamas 0–0,8 1/h rėžiuose, užduotą vertę keičiant kas 1–2 valandas.
2. Ištirpusio deguonies koncentracija buvo keičiama 0–20 procentų rėžiuose.
3. pH buvo palaikomas ties 7 verte.

Tiriant nuostato sekimą, buvo pasirinktas platus parametro kitimo diapazonas. Trikdžiai buvo parenkami taip, kad sukeltų maksimalų poveikį valdomam kintamajam siekiant imituoti ekstremalias darbo sąlygas.

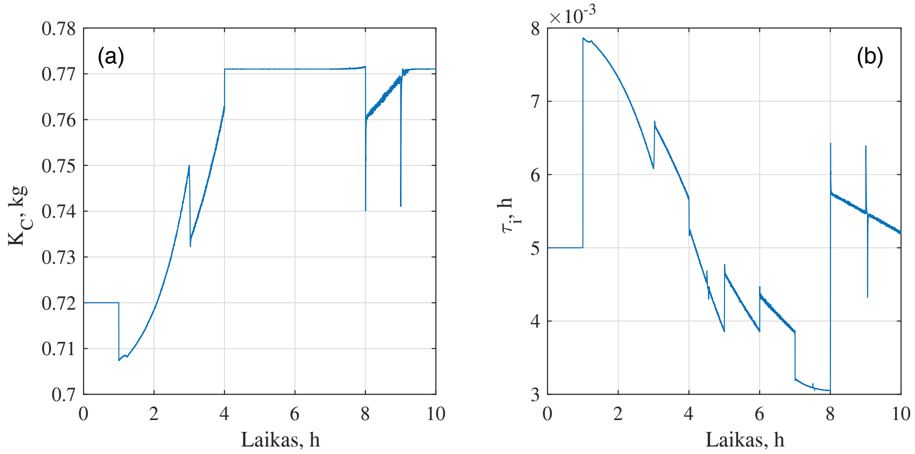
3.2. Neraiškiąja logika pagrįsto adaptyvaus santykinio augimo greičio valdymo efektyvumo vertinimas

Sukurto valdymo algoritmo efektyvumui įvertinti ir palyginti buvo pasirinktas ITAE kriterijus. Neraiškiąja logika pagrįstas adaptyvusis PI reguliatorius buvo lyginamas su [58] ištirtais stiprinimo numatymo ir MFA algoritmais, taikant tą patį biotechnologinio proceso matematinį modelį [106]. Sukurto neraiškiąja logika pagrįsto adaptyvaus PI reguliatoriaus valdymo efektyvumo pavyzdys pateiktas 3.2.1 pav. Sistema išlieka stabili plačiame užduotosios vertės diapazone. Modeliuojant OUR signalą naudotas adityvusis baltasis Gauso triukšmas. Tyrimas atliktas MATLAB aplinkoje.



3.2.1 pav. Santykinio augimo greičio (SGR) (sistemos išėjimo kintamasis) (a), substrato pamaitinimo greičio (reguliuojamas kintamasis) (b), deguonies suvartojimo greičio (OUR) (c) ir biomasės koncentracijos (d) kitimas simuliacijos metu

Sukurto adaptyvaus PI reguliatoriaus derinimo parametrų pokytis laike pavaizduotas 3.2.2 pav. Reguliatoriaus stiprinimo parametras K_c laikui bėgant pasikeičia maždaug 10 procentų tik dėl to, kad šis parametras koreliuoja su kultūros svoriu w, kuris imituojamo proceso metu padidėjo nedaug. Integravimo laiko konstanta T_i atitinka OUR profilio pokyčius, todėl atspindi gerokai kintančią proceso dinamiką.



3.2.2 pav. Stiprinimo koeficiento K_c (a) ir integralinės laiko pastovosios T_i (b) kitimas simuliacijos metu

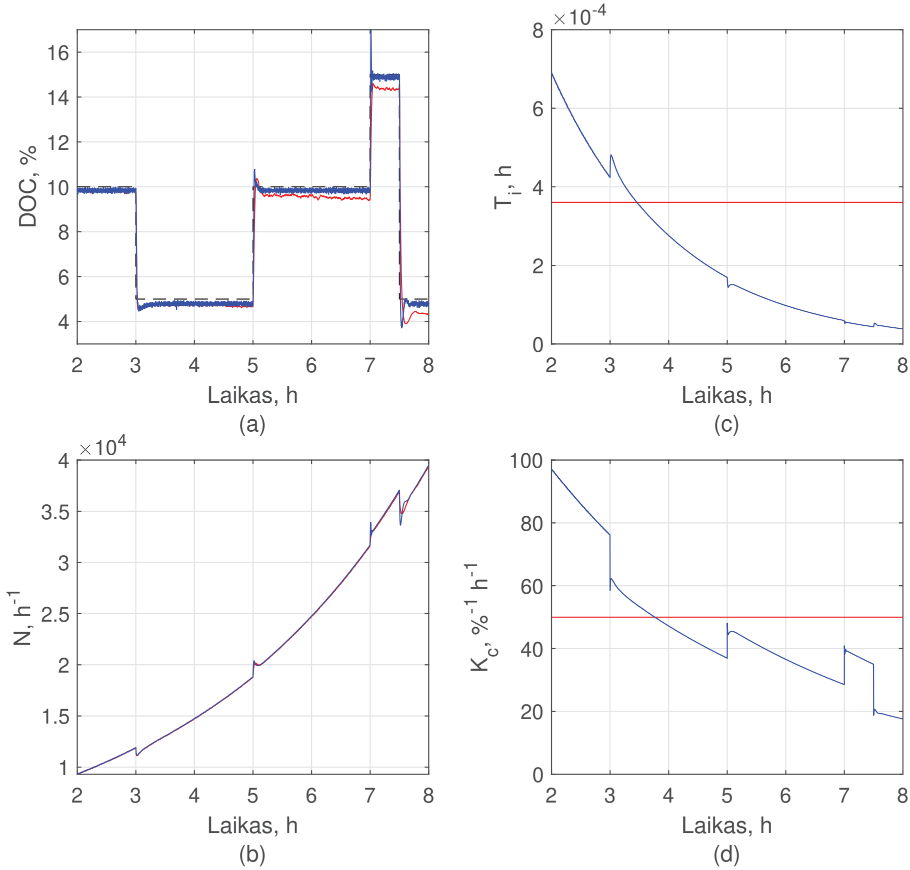
Visų metodų efektyvumo vertinimo suvestinė pateikta 3.2.1 lentelėje. ITAE vertės rodo, jog neraiškiaja logika pagrįstas adaptyvusis PI reguliatorius geba kompensuoti santykinio augimo greičio sumažėjimo sukeliamus trikdžius ir neatsilieka nuo stiprinimo numatymu ar MFA paremtų modelių. Šis metodas labiausiai tinka trikdžių kompensavimo valdymo sistemoms.

3.2.1 lentelė. Algoritmų rezultatų palyginimas

Valdymo tipas	ITAE		
	MFA modelis	GS modelis	*Fuzzy* modelis
Užduotos vertės sekimas	0,6865	0,6592	0,6723
Trikdžių kompensavimas	0,3783	0,3962	0,3431
Užduotos vertės sekimas ir trikdžių kompensavimas	0,7598	0,7357	0,7349

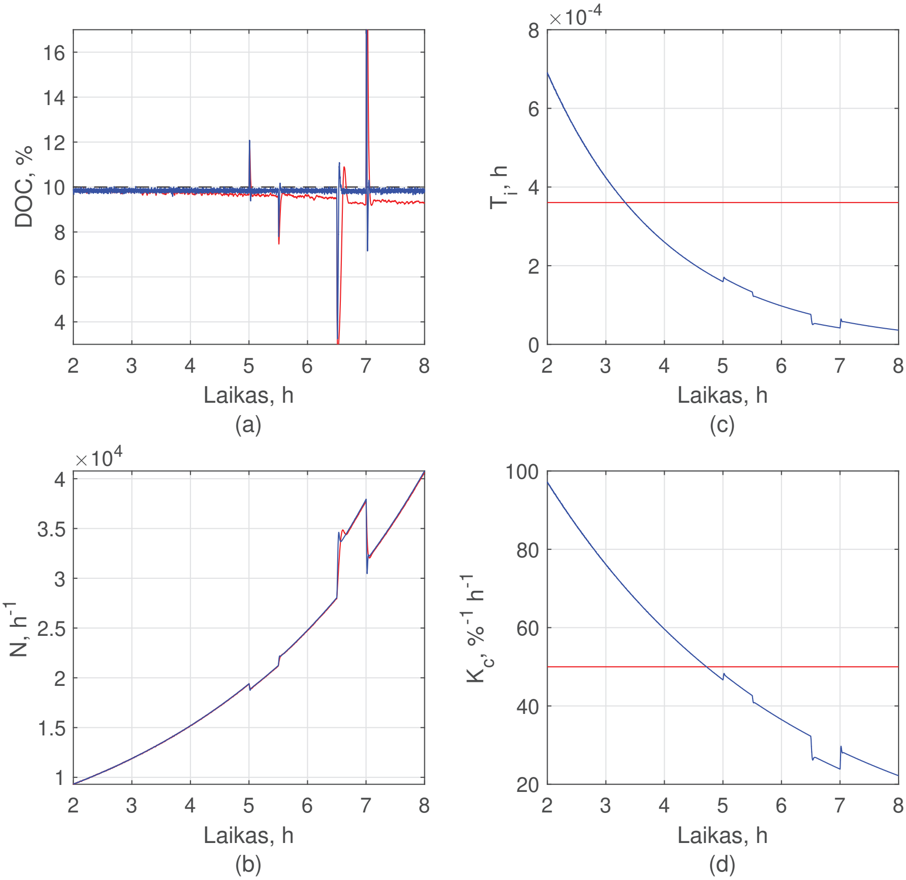
3.3. Stiprinimo numatymu paremto ištirpusio deguonies koncentracijos valdymo efektyvumo vertinimas

Kultivavimo proceso metu ištirpusio deguonies koncentracija buvo valdoma PI reguliatoriumi. Valdymui įvertinti buvo lyginami PI reguliatoriai su pastoviais ir stiprinimo numatymo principu adaptuojamais reguliatoriaus parametrais. Maišymo greitis N buvo pasirinktas kaip valdymo kintamasis. Modelio parametrai pateikiami [25, 106, 103]. Pirmiausia buvo ištirtas adaptyviosios valdymo sistemos veikimas, siekiant sekti nustatytąją vertę. Atliekant imitacinius eksperimentus MATLAB/Simulink aplinkoje, buvo pasirinktas paveiksle pavaizduotas ištirpusio deguonies koncentracijos užduotosios vertės pokyčio laiko profilis, kad būtų galima imituoti tikroviškas proceso sąlygas.



3.3.1 pav. DOC (sistemos išėjimas) (a), T_i (c), maišymo greičio N (valdymo kintamasis) (b), ir K_c (d) trajektorijų kitimas laike. Nuostato sekimas: PI reguliatorius su pastoviais parametrais (raudona), adaptyvus PI reguliatorius (mėlyna); DOC nuostato signalas (juoda)

Siekiant įvertinti trikdžių kompensavimo efektyvumą, sistema buvo modeliuojama esant pastoviai 10 procentų nustatytai vertei. Trikdžiams imituoti buvo pasirinktas oro tiekimo greičio pokytis. Sistemos atsakas ir valdymo charakteristikos pavaizduoti 3.3.2a paveiksle. Valdomo maišymo greičio trajektorija pateikta 3.3.2b pav.



3.3.2 pav. DOC (sistemos išėjimas) (a), T_i (c), maišymo greičio N (valdymo kintamasis) (b), ir K_c (d) trajektorijų kitimas laike. Trikdžių kompensavimas: PI reguliatorius su pastoviais parametrais (raudona), adaptyvus PI reguliatorius (mėlyna); DOC nuostato signalas (juoda)

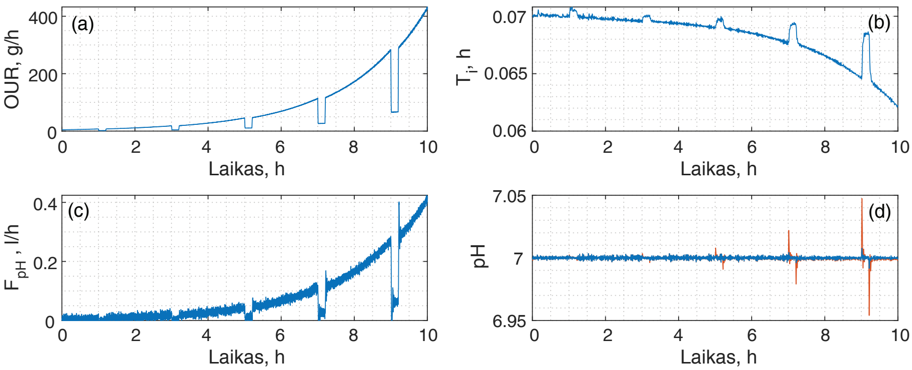
Modeliavimo rezultatai rodo, kad PI reguliatorius su stiprinimo numatymu užtikrina gerą DOC valdymo kokybę plačiame nuostato kitimo diapazone bei kompensuojant trikdžius ir akivaizdžiai lenkia įprastinį PI reguliatorių. Integravimo laiko konstanta T_i ir reguliatoriaus stiprinimas K_c keitėsi plačiame diapazone, todėl atspindi kintančią proceso dinamiką. Modeliavimo rezultatų analizė rodo, kad adaptyvioji sistema sumažino tirtų valdymo schemų vidutinę absoliutinę paklaidą daugiau kaip 2 kartus. Pereinamųjų procesų, kuriuos sukėlė užduotosios vertės pokytis, trukmė adaptyviojoje sistemoje buvo maždaug 2 kartus trumpesnė. Tirtų sistemų valdymo efektyvumas apibendrintas 3.3.1 lentelėje. Pagal vidutinę absoliutinę paklaidą adaptyvusis valdymo algoritmas standartinę sistemą lenkia maždaug 2 kartus.

3.3.1 lentelė. Reguliatoriaus parametrai ir ištirtų sistemų efektyvumo palyginimo rezultatai

Valdymo tipas	Reguliatoriaus parametrai	Vidutinė absoliutinė paklaida	
		Trikdžių kompensavimas	Nuostato sekimas
Standartinis DOC	$K_c = 50\%^{-1}h^{-1}$, T_i = 3e-4 h	0,1660	0,0710
Adaptyvus DOC	K_{Ti} = 0,6e5, K_{Kc} = 1,5e5	0,0630	0,0280

3.4. PI reguliatoriaus parametrų adaptavimo, remiantis grįžtamojo ryšio signalų statistine analize, valdymo efektyvumo vertinimas

Siekiant įvertinti modelio efektyvumą palaikant pastovią pH 7 vertę, sukurtas algoritmas palygintas su standartiniu PI reguliatoriumi, kurio parametrai palaikomi pastovūs. Imitaciniai eksperimentai buvo atliekami MATLAB/Simulink programiniu paketu. Bazės tirpalo (šarmo) padavimo srauto greitis F_{pH} buvo pasitinktas kaip valdymo kintamasis. Modelio parametrai pateikiami [103]. Modeliavimo rezultatai rodo, kad tiriamas pH valdymo algoritmas su tinkamai parinktomis derinimo parametrų reikšmėmis užtikrina patikimą reguliatoriaus integravimo parametro T_i adaptavimą ir stabilų veikimą. Modeliavimo rezultatai, įskaitant OUR (3.4.1a pav.), šarmo padavimo srauto (3.4.1c pav.) ir reguliatoriaus derinimo parametro T_i adaptacijos (3.4.1b pav.) trajektorijas, pateikti 3.4.1 paveiksle.



3.4.1 pav. Adaptyvi valdymo sistema: (a) OUR laiko profilis, (b) T_i parametro adaptavimas, (c) padavimo srauto greitis F_{pH} (valdomas kintamasis), (d) adaptyvios (mėlyna) ir standartinės (raudona) valdymo sistemos palyginimas

Adaptyvioji valdymo sistema geriau veikė kompensuojant trikdžius, kur adaptyvusis reguliatorius IAE kriterijų sumažino beveik 2,5 karto (3.4.1 lentelė).

3.4.1 lentelė. Standartinės ir adaptyvios valdymo sistemų efektyvumo palyginimo rezultatai

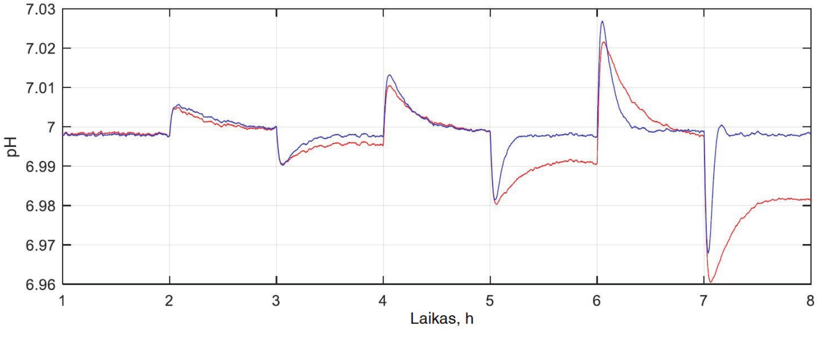
Valdymo tipas	IAE	
	Standartinis PI	Adaptyvus PI
Trikdžių kompensavimas	0,0041	0,0017
Nuostato sekimas ir trikdžių kompensavimas	0,0126	0,0099

3.5. Stiprinimo numatymu pagrįstos adaptyvios pH valdymo sistemos efektyvumo vertinimas

Sukurtos adaptyviosios pH valdymo sistemos veikimas buvo ištirtas atliekant kompiuterinį modeliavimą MATLAB/Simulink programine įranga. Modelyje santykinis augimo greitis buvo palaikomas pastovus, taip imituojant artimas realioms darbo sąlygas. Sistemos gedimas buvo imituojamas santykinį augimo greitį sumažinant nuo 0,5 iki 0,25 1/h kas 2 valandas. Šarmo tirpalo padavimo srauto greitis buvo pasirinktas kaip valdymo kintamasis. Simuliacijoje naudoto matematinio modelio parametrai pateikiami [103]. Atlikus preliminarius eksperimentus buvo nustatytos fiksuotos derinimo parametrų vertės ($K_c = -3,3 \cdot 10^6$ h/l, T_i = 0,3 h), kurios užtikrina patenkinamą pH valdymo tikslumą ir stabilų F_{pH} signalą (be didelių svyravimų) laikui bėgant.

3.5.1. Adaptyvios pH valdymo sistemos efektyvumo vertinimas kultivacijos metu matuojant V ir F_s vertes

Kaip matyti iš lygčių (2.9) ir (2.10), PI reguliatoriaus reguliavimo parametrai K_c ir T_i priklauso nuo reguliatoriaus įėjimo ir išėjimo parametrų C_{H^+} bei F_{pH}, substrato pamaitinimo greičio F_s ir kultūros tūrio V. Žemiau pateiktas grafikas atvaizduoja sukurtos sistemos ir standartinės sistemos veikimą, kai minėti parametrai matuojami kultivacijos metu:



3.5.1 pav. Adaptyvios (mėlyna) ir standartinės (raudona) pH valdymo sistemos kokybės palyginimas

3.5.2. Adaptyvios pH valdymo sistemos efektyvumo vertinimas kultivacijos metu matuojant F_s vertę

Siekiant sumažinti sistemos sudėtingumą, adaptyvus valdymo algoritmas supaprastinamas darant prielaidą, kad terpės tūris proceso metu kinta nedaug, ir vietoje realios reikšmės naudojama vidutinė vertė:

$$T_i = \frac{k_{Ti} V_{avg}}{F_{pH} + F_s}, \tag{2.20}$$

$$K_c = \frac{K_{Kc} V_{avg}}{C^0_{H^+} - C_{H^+}}. \tag{2.21}$$

Ši sistema šiek tiek padidina ITAE vertę, kai sekama užduotoji vertė, bet šiek tiek sumažina, kai trikdžiai kompensuojami. Pasiūlyta adaptyvioji sistema (toliau vadinama C sistema) vėlesniuose proceso etapuose geriau susidoroja su trikdžiais nei standartinis PI reguliatorius.

3.5.3. Adaptyvios pH valdymo sistemos efektyvumo vertinimas kultivacijos metu matuojant V vertę

Kadangi substrato suvartojimo greitis F_s yra apytiksliai proporcingas pH reguliatoriaus išėjimo signalui (valdymo kintamasis F_{pH}), galima daryti prielaidą, kad:

$$F_s = kF_{pH}, \tag{2.22}$$

čia k yra derinimo parametras, kuris turi būti identifikuotas. Tokia supaprastinta valdymo sistema (toliau vadinama D sistema) veikė panašiai, kaip anksčiau aprašyta C sistema, ir gali būti naudojama kaip alternatyva, jei substrato padavimo greitis nėra matuojamas kultivacijos metu.

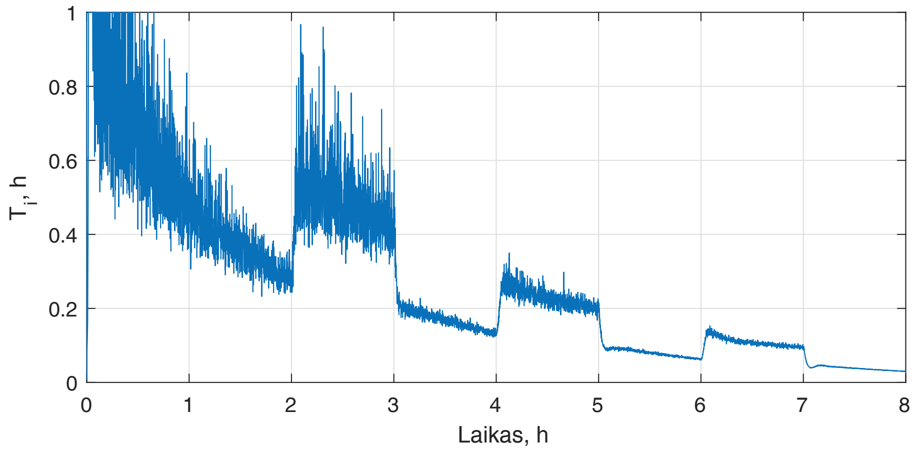
3.5.4. Adaptyvios pH valdymo sistemos efektyvumo vertinimas naudojant tik reguliatoriaus įėjimo ir išėjimo parametrus

Patraukliausias PID (PI) reguliatoriaus parametrų stiprinimo numatymas yra pagrįstas tik reguliatoriaus įvesties ir išvesties signalais, todėl nereikia papildomai matuoti proceso kintamųjų, kad būtų galima adaptuoti reguliatoriaus parametrus. Kadangi reguliatoriaus parametrai K_c ir T_i priklauso nuo terpės tūrio V, bazės bei substrato srautų F_{pH}, F_s ir vandenilio jonų koncentracijos C_{H^+}, tokiu atveju reguliatoriaus parametrų adaptacija gali būti aprašyta:

$$T_i = \frac{K}{F_{pH}}, \tag{2.23}$$

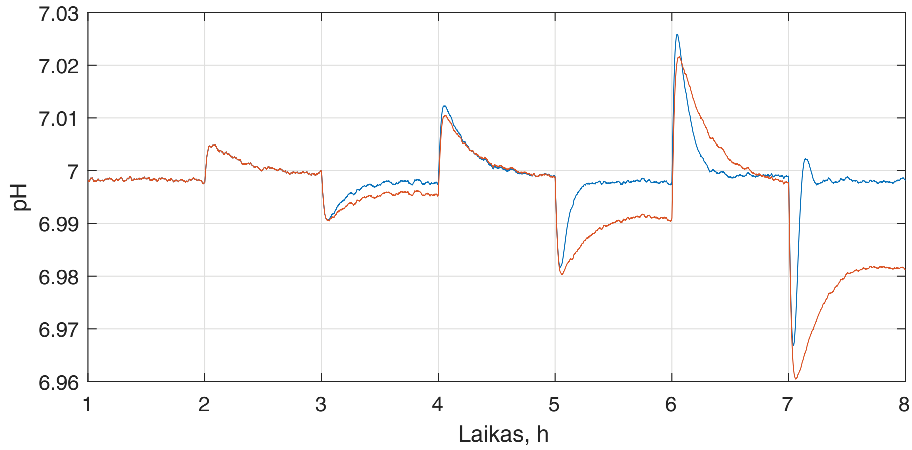
$$K_c = \frac{C}{C^0_{H^+} - C_{H^+}}, \tag{2.24}$$

čia $K = \frac{k_{Ti} V_{avg}}{k}$ ir $C = K_{Kc} V_{avg}$ yra reguliavimo parametrai. Pasiūlytas adaptyvus valdymo algoritmas veikia geriau nei PI reguliatorius su pastoviais parametrais, tačiau modeliavimo rezultatai rodo, kad proceso pradžioje T_i smarkiai svyruoja.



3.5.2 pav. Reguliatoriaus derinimo parametro T_i kitimas laike

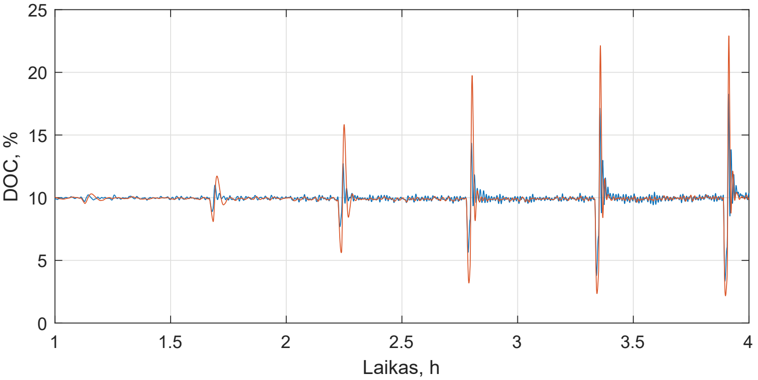
Sistemos stabilumui užtikrinti rekomenduojama adaptyvų valdymą įjungti antroje proceso pusėje. Simuliacijos rezultatai pateikti 3.5.3 paveiksle.



3.5.3 pav. Adaptyvios (mėlyna) ir standartinės (raudona) pH valdymo sistemos kokybės palyginimas

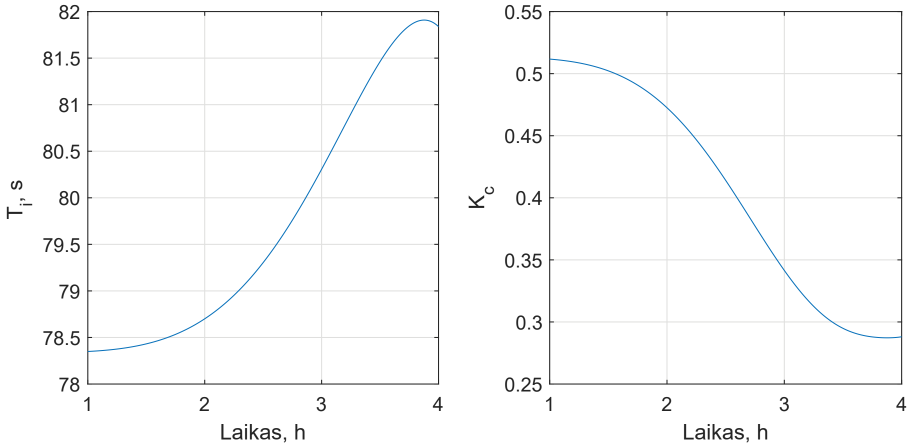
3.6. Adaptyvaus DOC valdymo algoritmo netipiniuose kultivavimo procesuose efektyvumo vertinimas

Sukurtas valdymo algoritmas buvo lyginamas su PI reguliatoriumi, kurio parametrai laike išlieka pastovūs. Modeliavimo eksperimentai buvo atlikti MATLAB/Simulink aplinkoje. Matematinio modelio parametrai pateikiami [25, 85]. Šiame tyrime maišymo greitis N buvo pasirinktas kaip valdymo kintamasis. Adaptyvusis valdymo algoritmas buvo pranašesnis už standartinę valdymo sistemą kompensuojant trikdžius. Modeliuojamų sistemų DOC pokyčio palyginimas pateiktas 3.6.1 pav.



3.6.1 pav. Adaptyvios (mėlyna) ir standartinės (raudona) DOC valdymo sistemos kokybės palyginimas

PI reguliatorius su pastoviomis K_c ir T_i reikšmėmis buvo derinamas Zieglerio ir Nikolso metodu. Adaptyvioji valdymo sistema 20 procentų sumažino sistemos IAE ir geriau kompensavo trikdžius. Reguliatoriaus parametrų kitimas laike pateiktas 3.6.2 paveiksle:



3.6.2 pav. Reguliatoriaus parametrų kitimas laike

3.7. Pamaitinimo profilių adaptavimo, panaudojant OUR ir substrato tiekimo srautu pagrįstą rodiklį, efektyvumo vertinimas

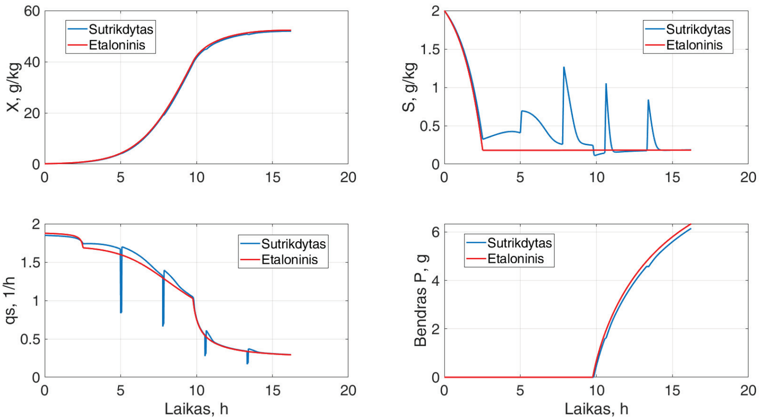
Sukurto substrato tiekimo valdymo algoritmo efektyvumas buvo patikrintas atliekant imitacinius tyrimus MATLAB/Simulink aplinkoje ir realius eksperimentus KTU laboratorijoje. Modeliavimo tikslas – imituoti realaus auginimo proceso elgseną interferono alfa 5 (IFNa5) baltymui gaminti, išreiškiant jį rekombinantinėje *E.coli* bakterijoje, gaminančioje IFNa5 [125, 107].

Buvo atlikti limituoti auginimo eksperimentai su įvairiais gliukozės pamaitinimo scenarijais. Pamaitinimo profiliai su įvairiais gliukozės suvartojimo greičio apribojimais generuojami pratęsiant auginimo laiką atvirkščiai proporcingai apribojimo lygiui, kol pasiekiamas neribojančio eksperimento metu užfiksuotas suvartotos gliukozės kiekis. Remiantis šių eksperimentų rezultatais, turi būti parinktas etaloninis gliukozės pamaitinimo profilis $Fgl_{etaloninis}$, kuris užtikrina pakankamą proceso stabilumą, pakartojamumą ir didelę tikslinių baltymų gamybą. Etaloniniam procesui taip pat turi būti registruojamas deguonies suvartojimo greitis ir nustatomas deguonies suvartojimo iš paduodamos gliukozės išeigos profilis $Y_{OUR/Fgl_{etaloninis}}$.

Pasirinktą etaloninį gliukozės pamaitinimo profilį rekomenduojama taikyti realiuose auginimo procesuose. Pagal pasiūlytą gliukozės limitavimo metodą sukurtas etaloninis pamaitinimo profilis yra pakankamai patikimas, o tipiniai trumpalaikiai proceso sutrikimai neturi įtakos proceso kokybei. Esant didesniems trikdžiams, ypač kai maksimalus savitasis gliukozės suvartojimo greitis sumažėja daugiau kaip 5–8 %, auginimo procesas gali tapti nestabilus ir neefektyvus dėl bioreaktoriuje susikaupusio didelio gliukozės kiekio. Sukurtam valdymo algoritmui vertinti pasirinktas substrato tiekimo režimas (etaloninis procesas), kai gliukozės limitavimo lygis yra 90 %. Šiuo atveju *E.coli* kultivavimo procesas yra patikimas, todėl tipiniai trumpalaikiai proceso trikdžiai (santykinio gliukozės suvartojimo greičio sumažėjimas 30 % per 15 minučių įvairiuose kultivavimo proceso etapuose) neturi įtakos kultivavimo proceso kokybei. Tikslinio baltymo P kiekis ($P = 0,01 p_x xw$) tokiu kultivavimo atveju buvo maždaug toks pat, kaip ir etaloninio proceso atveju, $P = 6,32g$.

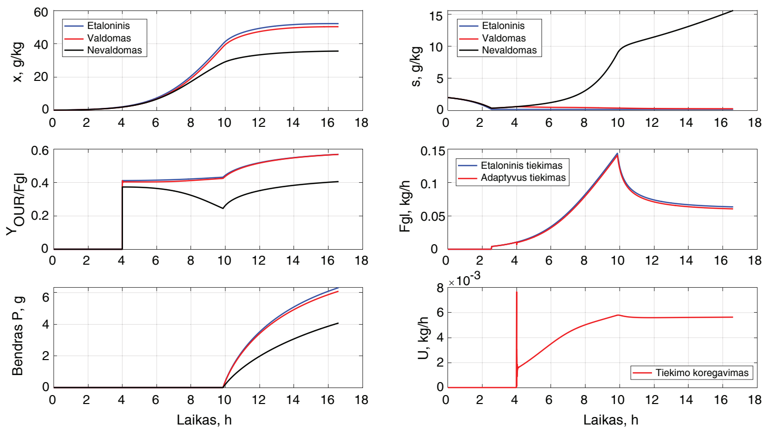
Tokie trikdžiai yra būdingi realiuose kultivavimo procesuose, ypač kai į bioreaktorių įlašinamas putojimą mažinantis skystis. Tai gali smarkiai sumažinti gliukozės suvartojimą ir biomasės augimo greitį, tačiau dėl maitinimo profilio atsparumo tai reikšmingos įtakos kultivavimo procesui neturi, jei santykinis gliukozės suvartojimas sumažėja ne daugiau nei 3 %.

Galima daryti išvadą, kad, veikiant nedideliems trikdžiams, maitinimo profilio adaptavimas nėra būtinas, jei profilis sudarytas prieš tai aprašymu būdu, o sisteminiai trikdžiai nedideli. O tai itin svarbu pramoniniuose kultivavimo procesuose, kadangi bet kokios papildomos procesų korekcijos reikalauja sudėtingų proceso taisyklių ir receptų pakeitimo.



3.7.1 pav. Biomasės x, substrato s, substrato suvartojimo greičio q_s koncentracijų bei bendro produkto kiekio P kitimas sutrikdytame ir nesutrikdytame procese

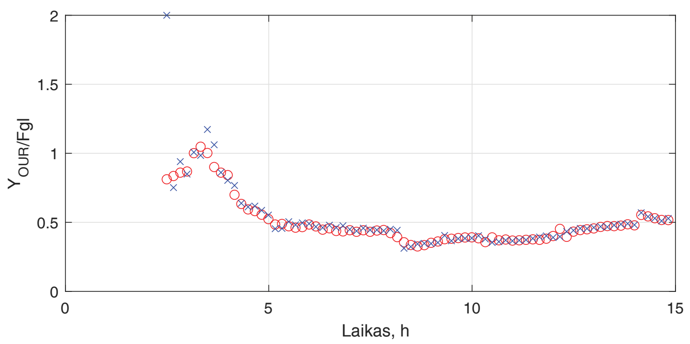
Tačiau pramoniniuose kultivavimo procesuose gali pasitaikyti ir didesnių trikdžių, kurių atveju etaloninis maitinimo profilis nepajėgia užtikrinti proceso stabilumo dėl perteklinio gliukozės kiekio. Tokio trikdžio pavyzdys būtų maksimalaus santykinio gliukozės suvartojimo sumažėjimas daugiau nei 5 procentais, kuris nulemtų gliukozės kaupimąsi ir pagaminto produkto sumažėjimą, ar, pavyzdžiui, trūkusi substrato tiekimo žarnelė.



3.7.2 pav. Biomasės x, substrato s, adaptacijos rodiklio $Y_{OUR/Fgl}$, substrato tiekimo Fgl, produkto P ir tiekimo koregavimo signalo U kitimas laike

Tokiems atvejams yra numatytas kultivavimo proceso valdymo žingsnis netiesiogiai stebėti gliukozės kaupimąsi kultivavimo terpėje ir kultivacijos metu adaptuoti etaloninį gliukozės maitinimo profilį. Šiuo tikslu kultivacijos metu atliekamas deguonies sunaudojimo tiekiamai gliukozei santykio vertinimas, naudojant slenkančio lango metodą (30s langas), kuris lyginamas su etaloniniu indikatoriumi. PI reguliatoriumi etaloninis gliukozės suvartojimo profilis yra adaptuojamas. Šis etaloninio profilio adaptavimo žingsnis leido pašalinti perteklinį gliukozės kaupimąsi bioreaktoriaus terpėje ir pagerino tikslinio baltymo gamybos efektyvumą. Profilio adaptavimo žingsnis padėjo išvengti per didelio gliukozės kaupimosi ir pagerino proceso kokybę. Pagaminto tikslinio baltymo kiekis buvo artimas etaloniniam (5,9 g ir 6,3g atitinkamai). O sutrikdytame procese, kuriame adaptavimas nebuvo taikomas, tikslinio produkto kiekis pasiekė tik 4,3g.

Šio metodo veikimas buvo testuojamas eksperimentiškai. Pirmojo eksperimento metu buvo nustatytas etaloninis substrato pamaitinimo ir $Y_{OUR/Fgl}$ profilis kultivacijos metu matuojant OUR ir Fgl vertes. Kultivacijos metu apskaičiuotos indikatoriaus $Y_{OUR/Fgl}$ vertės buvo palygintos su teorinėmis indikatoriaus vertėmis modeliuotame procese (matematinio modelio parametrai, naudoti simuliacijoje, pateikiami pagrindinio teksto 2.11 lentelėje).



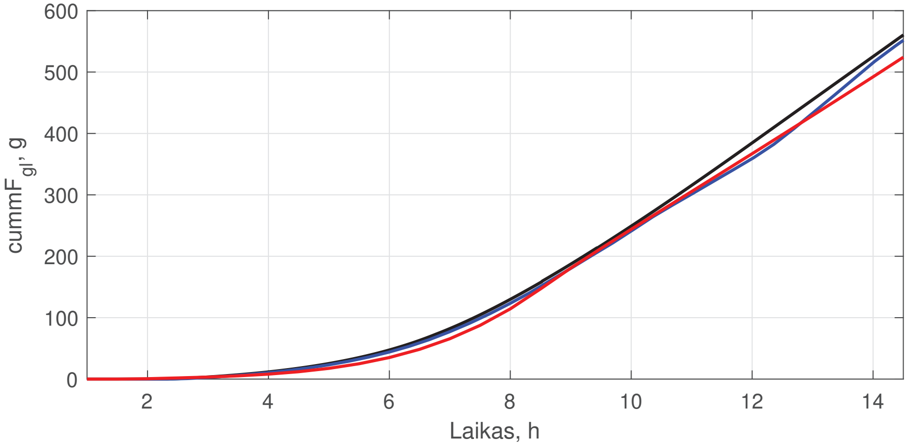
3.7.3 pav. Eksperimento metu gautų $Y_{OUR/Fgl}$ (mėlyna) ir modeliuotų $Y_{OUR/Fgl}$ (raudona) verčių palyginimas

Eksperimentų rezultatai pateikti 3.7.1 lentelėje.

3.7.1 lentelė. Vidutinės absoliutinės ir kvadratinės paklaidos palyginimas

Eksp. numeris	Vidutinė absoliutinė paklaida	Vidutinė kvadratinė paklaida
1	0,0110	0,0230
2	0,0230	0,0580
3	0,0220	0,0790

Vidutinės absoliutinės paklaidos vidurkis buvo 0,0180, o vidutinės kvadratinės paklaidos vidurkis siekė 0,0530. Eksperimentiniai rezultatai patvirtina, kad šis indikatorius yra tinkamas substrato padavimo profiliui adaptuoti biotechnologiniuose kultivavimo procesuose. Sukurtas algoritmas geba reaguoti į trikžius ir kompensuoti jų poveikį (substrato žarnelės trūkimas vieno iš eksperimentų metu), taip užtikrinant sklandų veikimą. Etaloninio ir adaptuoto profilio kitimo kreivių palyginimas pateiktas 3.7.4 paveiksle.



3.7.4 pav. Pamaitinimo profilio adaptavimas. Juoda linija atvaizduoja etaloninį profilį, mėlyna – eksperimento metu gautą adaptuotą profilį, raudona – modeliuotą adaptuotą profilį

Iš grafiko matyti, jog kultivacijos metu algoritmas adaptuoja etaloninį pamaitinimo profilį, taip reaguodamas į besikeičiančius proceso parametrus ar trikdžius. Simuliacijos ir eksperimentų rezultatai patvirtina būtiną salygą modeliui verifikuoti. Dėl didelių eksperimentų atlikimo kaštų planuojama modelio validavimą atlikti ateityje.

IŠVADOS

1. Sukurtas neraiškiąja logika pagrįstas adaptyvaus valdymo algoritmas, kompensuojant nuokrypius, pranoksta kitus adaptyviuosius metodus. ITAE reikšmė buvo sumažinta 9 % kompensuojant trikdžius ir 0,11 % – sekant užduotąją vertę ir kompensuojant trikdžius, todėl šis algoritmas tinka periodiniams su pamaitinimu biotechnologiniams procesams valdyti.

2. Vidutinė absoliutinė paklaida sumažinta daugiau nei 60 % kompensuojant trikdžius ir sekant užduotą signalą tiek pH, tiek DOC valdymo atveju, naudojant stiprinimo numatymu pagrįstus adaptyvius valdymo algoritmus. Naudojant tik reguliatoriaus įvesties ir išvesties kintamuosius, valdymo algoritmui sukurti nereikia atlikti papildomų proceso kintamųjų matavimų realiu laiku. Tai neišplečia valdymo algoritmui įgyvendinti reikalingos įrangos kiekio.

3. Polinomais pagrįstas adaptyvus valdymo algoritmas sumažino ISE vertę 3 kartus, o grįžtamojo ryšio statistine analize paremtas adaptyvus valdymo algoritmas 2,5 karto. Bazinėmis matematinėmis funkcijomis paremtas PI(D) reguliatoriaus parametrų perskaičiavimas leidžia šiuos adaptyvius algoritmus įdiegti pramoniniuose valdikliuose.

4. Gliukozės kaupimosi periodiniuose su pamaitinimu procesuose galima išvengti matuojant OUR ir substrato tiekimo srautu pagrįstą rodiklį, kuris naudojamas substrato padavimo srautui adaptuoti kultivacijos metu. Substrato padavimo srauto adaptavimas sumažino galutinio produkto nuostolius nuo 37% nevaldomame procese iki 3,8%, taip išvengiant substrato kaupimosi.

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1. IVUS 2019 24^{th} International Conference on Information Technologies. Mathematical model library for recombinant e.coli cultivation process. Kaunas, Lithuania, April 25, 2019.

2. ICHEAP15, 15^{th} International Conference on chemical and process engineering. Unified Structure of Adaptive System for Control of Basic Process Variables in Biotechnological Cultivation Processes: pH Control System Case Study. Presented virtually. Naples, Italy, May 23-26, 2021.

3. CASE 21 IEEE 17^{th} International Conference on Automation Science and Engineering. Investigation of Gain Scheduling Algorithm for Controller Adaptation in Microbial Cultivation Process Control Systems. August 23-27, 2021, Lyon, France.

Patents registered by the State Patent Bureau of the Republic of Lithuania

1. Galvanauskas, Vytautas (autorius, išradimo); Simutis, Rimvydas (autorius, išradimo); Levišauskas, Donatas (autorius, išradimo); Urniežius, Renaldas (autorius, išradimo); Vaitkus, Vygandas (autorius, išradimo); Tekorius, Tomas (autorius, išradimo); **Butkus, Mantas** (autorius, išradimo); Survyla, Arnas (autorius, išradimo). Maitinimo profilių, skirtų valdyti pusiau-periodinius rekombinantinių E. coli kultivavimo procesus, formavimo ir adaptavimo būdas / išradėjai: V. Galvanauskas, R. Simutis, D. Levišauskas, R. Urniežius, V. Vaitkus, T. Tekorius, **M. Butkus**, A. Survyla; savininkas: Kauno technologijos universitetas. LT 6861 B. 2021- 11-10.

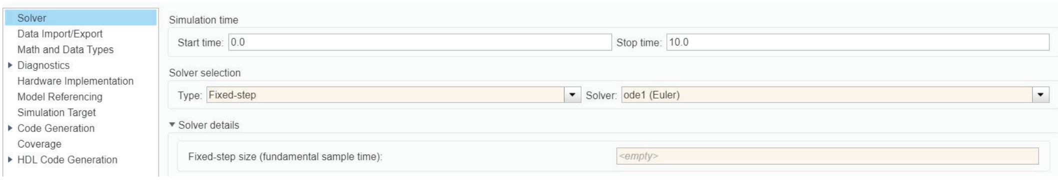
Patent applications

1. Method for design and adaptation of feeding profiles for recombinant e.coli fed-batch cultivation processes / inventors: Galvanauskas, Vytautas; Simutis, Rimvydas; Levišauskas, Donatas; Urniežius, Renaldas; Vaitkus, Vygandas; Tekorius, Tomas; **Butkus, Mantas**; Survyla, Arnas; applicant: Kaunas University of Technology. EP4083185 (A1). 2022-11-02. [Espacenet]

APPENDIXES

Appendix 1. MATLAB/Simulink initialization

In this doctoral dissertation, numerical simulations were carried out by using *Matlab/Simulink*. To calculate the differential equations, the Euler's method was used that is represented by the ode1 function in *Matlab*. In *Simulink*, the ode1 can be selected in the modeling parameters:



The simulation duration and step size is selected based on the modeled process. Time discretization is set to 0.18 s. Relative tolerance for the integration routine is set to 1e-6. This tolerance measures the error relative to the magnitude of each solution component. A pseudo-code example for the modeling of a biotechnological process is presented bellow:

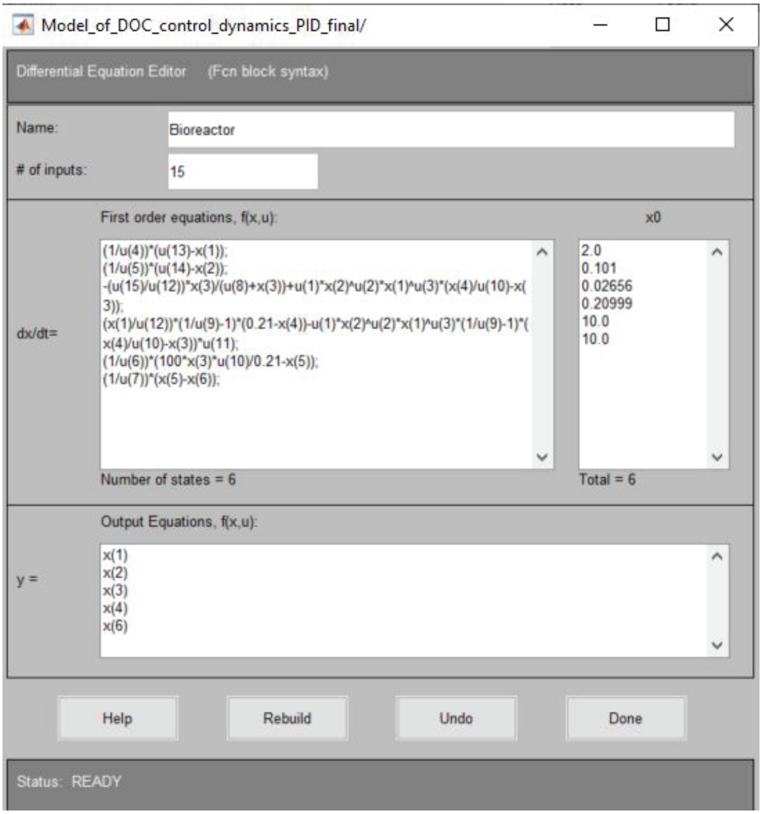
% Declaration of global parameters
<Global parameters>
% Parameters needed for ODE numerical integration function
eps=1e-6;
options = odeset('RelTol', eps);
% Model parameters
<Model parameter variables>
% Define process time and intial conditions
<process time and intial conditions variables>
% Call of ODE numerical integration function
[t,c] = ode1(@model,tspan,c0,options);
function dcdt=model(t,c)
% Redefinition of the state variables for convenience
<State variables>
% Define growth rates
<specific reaction rates>
% Define feeding profiles
<Feeding profiles>
% Differential equations
<Differential equations>

Appendix 2. Simulink DEE block set-up

To solve differential Equations in Simulink, the Differential Equation Editor function block was used. As of 2020, this function block needs to be implemented separately in Matlab. When opened, the DEE block has the following available inputs:

1. Name
2. Number of inputs
3. First order equations f(x,u)
4. Initial conditions for the state variables
5. Output equations

The differential equations of the biotechnological process are entered in the first order equations field, where x(n) represents the n^{th} state variable, and u(n) represents the n^{th} DEE block input. An example of the DEE block for a biotechnological process is presented in the following figure.



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