Adaptive Control of *Saccharomyces cerevisiae* Yeasts Fed-Batch Cultivations

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In this paper, the application of an adaptive algorithm for control of fed-batch bioprocess capable of coping with time-variant process properties in the presence of uncertainties is introduced. The proposed adaptive controller uses Maršík's heuristic algorithm for adaptation based on control error oscillation rate criterion without the need of a mathematical model of the controlled process or any special test signals. The intended application of the resulting controller was off-gas CO_2 concentration control in fed-batch yeast cultivations where the set point has the form of a time-varying concentration profile. The controller has been tested in a series of experimental fed-batch cultivations with D7 *Saccharomyces cerevisiae* strain, a UV mutant suitable for ergosterol production, in 7-litre laboratory bioreactor. Obtained results demonstrate good properties of this adaptive controller that can be used without the need for a tedious parameter identification of the complex bioprocess.

Key words:
Adaptive control, identification-free, fed-batch cultivation, baker's yeast, and *Saccharomyces cerevisiae*

Introduction

Fed-batch baker's yeast cultivation is a complex biotechnological process from the viewpoint of measurement and control. It is a non-linear system with not well-known dynamics. The process is non-stationary due to metabolic changes, modifications in cell physiology and multiple increases in biomass concentration over the cultivation time. Furthermore, there is lack of cheap and reliable on-line sensors for measurement of important biochemical quantities, e.g. substrate and biomass concentration.[1] Because of the complexity and time variant nature of fed-batch cultivations, the use of PID controllers with constant parameters is restricted and modification of these parameters is often necessary during the cultivation.[2] This can be accomplished by an adaptive controller, which automatically adjusts its parameters to the actual state of the controlled process, using either a mathematical model of the process – e.g. internal model control principle[3] –, or an identification-free algorithm extracting information from the process data in real time as in this work.

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Reported model-based adaptive controllers for the control of bioprocesses include approaches based on Haldane kinetics,[4] adaptive-predictive controllers using a recursive least-square identification method for prediction consisting of an incremental linear model[5,6] or a model reference adaptive estimation and control using Lyapunov's method applied on a pilot-plant fermenter.[7] Further there is an application in the lactic fermentation process, in which parameters are estimated on-line and an adaptive-multivariable predictive controller is used.[8] Another application to an anaerobic digestion pilot plant introduces non-linearity in the control scheme in order to compensate non-linearity of the system.[9] A pole-placement control for time-varying multivariable first order plants[10] and a pole-assignment method in conjunction with ARMAX structured process model[11] can also be used. Other method is a discrete-time adaptive LQ control law,[12] an adaptive controller employing the linearized Kalman filter for the state estimation[13,14] or the concept of adaptive regulation based on respiratory quotient (RQ).[15,16] Alternatively, neural-network-based adaptive controllers exploiting learning capabilities of the artificial neural nets[17,18,19,20] and fuzzy relational predictive controllers with a fuzzy relation model[21] are used for the control of bioprocesses. Adaptive linear control strategies can be used for the optimal control of biotechnological processes with a yield–productivity conflict.[22,23] Recently, adaptive controllers are also frequently based on model predictive approach. The model is either in the form of a neural network,[20] neuro-fuzzy piecewise linear model,[24] non-linear model[25] or the alternative to non-linear model – the bond graph model.[26] Alternative methods using high gain robust control and non-linear observer[27] are used as well.

There are also approaches, which are not based on mathematical models. For instance, the geometric adaptive-predictive control algorithm for control of the dissolved oxygen concentration in fermentation processes proposed by Gomes & Menawat uses information contained in the shape (geometry) of the profile of the state variables as they evolve in time for adaptation to process variations and may be considered as model independent.[28] A promising method is the so-called probing control for tuning of the controller gain based on superimposing of a probing signal on the feed rate, which enables feeding at the critical glucose uptake.[29] Another example is Maršík's algorithm[30,31] that is based on the automatic tuning of the PID type controller parameters following oscillation rate criterion calculated exclusively from the control error. Maršík's heuristic adaptive controller is considered one of the successful adaptive control applications.[32] In the field of biotechnology it was applied for control of continuous cultivations of *Candida utilis* yeast.[33]

The aim of this work was to apply the Maršík's identification-free adaptive algorithm for fed-batch processes and to implement it into the BIOGENES II knowledge-based computer control system[34,35] as an optional regulatory strategy for the control of a selected model process – fed-batch cultivation process of *Saccharomyces cerevisiae*. The resulting adaptive controller named CO2MAR was used for maintaining the off-gas carbon dioxide (CO_2) concentration as a controlled variable on precalculated values of the CO_2 concentration set point by adjusting medium feed rate as a manipulated variable during yeast's cultivation. The set point profile of the CO_2 concentration was calculated online by a supervisory level of the knowledge-based system on the basis of the current metabolic state that was continuously inferred from a set of selected on-line measured and calculated process variables using knowledge about the yeast physiology. Details of the design of the supervisory knowledge-based system including the mechanism for the calculation of the CO_2 set point profile have been published previously by Hrnčiřík et al.[36] Alternatively, CO_2 set point profile could be calculated prior to the start of the process from a desired specific growth rate profile using mathematical model. This alternative mechanism for CO_2 set point profile calculation has been described and published previously by Rychtera et al.[37] A basic structure of the whole control system used in our studies is depicted in Fig. 1.

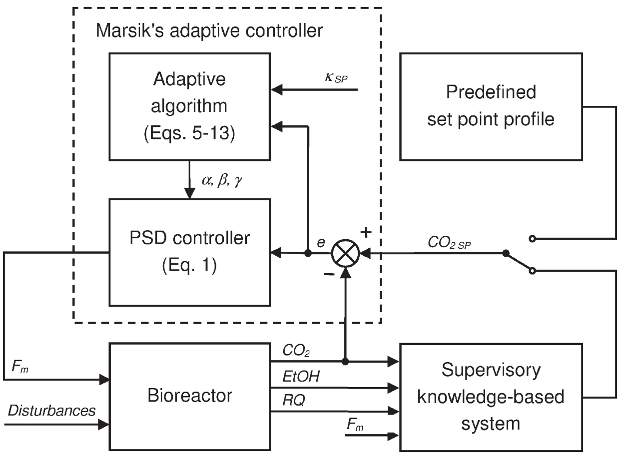

Fig. 1 – *Overall scheme of the adaptive control*

Materials and methods

Microorganism and cultivation

Saccharomyces cerevisiae, strain D7, prepared as a UV mutant giving better ergosterol biosynthesis, was provided by a yeast factory.[37]

An optimised synthetic medium consisting of glucose (125 g L^{-1}), yeast extract – DIFCO (31.2 g L^{-1}), $(NH_4)_2SO_4$ (7.8 g L^{-1}), KH_2PO_4 (3.7 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (3.1 g L^{-1}), $CaCl_2$ (1.25 g L^{-1}) dissolved in mains water was used. BREOX (solution of 5 vol %) was used as an antifoam agent and pH was controlled by adding 10 wt % NaOH and 10 wt % H_2SO_4 solutions at value 5. The temperature was kept constant in all experiments at 30 °C as well as the airflow (5 L min^{-1}) and stirrer speed (600 min^{-1}).

Instrumentation and software

The laboratory bioreactor with a volume of 7 L (manufactured by newMBR Switzerland) was used for the experiments. The bioreactor was equipped with an IMCS-2000 analogue control unit (PCS AG, Switzerland). The analogue control unit was used to stabilise the environmental conditions in the bioreactor: temperature, pH of the medium (Mettler Toledo probe), frequency of stirrer revolutions, air flow rate and foam level. Dissolved oxygen tension was also measured by a polarographic oxygen probe (Mettler Toledo). For supplying of cultivation medium to the bioreactor, a DP-200 peristaltic pump (New Brunswick Scientific) was used. SERVOMEX type 1100 and 1440 analysers with backpressure compensation and regulation and a gas sample conditioner (Baldwin) were used for measurement of the oxygen and carbon dioxide concentrations, respectively, in the outlet gas. The ethanol concentration in the outlet gas was measured by the METREX analyser, on-line biomass concentration analyser CELLEX provided turbidimetry and nephelometry measurements, both experimental analysers were constructed at the Institute of Chemical Technology, Prague.

All instruments were connected to a Compact (Schneider Electric) programmable logic controller, which provided all real-time measurement processing and control tasks. The Compact controller was connected via an OPC server to the proprietary BIOGENES II control system (based on Factory Suite 2000 software package, Wonderware, USA) running on PC.[34,35]

Maršík's identification-free algorithm for direct adaptation of controller parameters

Maršík's identification-free adaptive algorithm as a simple digital algorithm for direct heuristic adaptation of the parameters of the digital or analogue PID controllers is based on performance criterion using geometrical properties of the control error signal. The error signal is used for evaluation of the so-called oscillation rate criteria. This approach does not lead to a standard search for the extreme, thus the adaptation can be carried out as a standard feedback control as will be explained in the following sections. The algorithm needs no identification of the controlled system model and no special test signals.

The adaptation strategy is founded on the fact that optimal step responses of the controlled processes are fairly similar. They are different in terms of time and amplitude scale only. However, the shape of these transient responses is more or less the same, as though the control loop was the third or the second order system, even if controlled system is of higher order. Such a standard optimal response is not generally allowed to have more than one distinct overshoot. It corresponds to the course of the error with practically only two damped half-waves with all remaining oscillations negligible like in Fig. 2. The shape of more or less damped responses of this type, that is less or more oscillating, can be characterised by a value of the oscillation rate criterion. Therefore, the adaptation can be carried on so that the oscillation rate is kept at the value corresponding to the optimal response by tuning the gain coefficient of the controller.

This algorithm is suitable for the control of systems with arbitrary order, even with a non-minimal phase transfer function, linear or non-linear. It assumes that the controlled system is stable with monotonous or slightly oscillatory step response. Disturbances can be stochastic or deterministic.[30,31] The

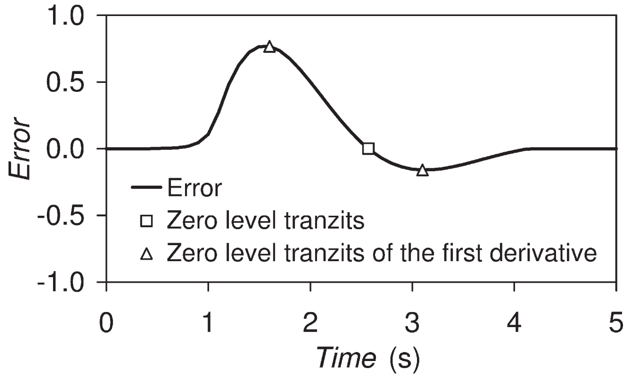

Fig. 2 – *Ideal response of a control loop with two half waves resulting in κ = 0.5*

controlled variable has to be free of high frequency noise. If the noise is not high then sampling corresponding to approx. 10 samples during the rising phase of the step response is sufficient. High frequency noise can also be removed by filtration of the control variable using e.g. moving average filter.

In the case of bioprocesses, these above listed conditions are generally satisfied with the exception of inherent system stability. Therefore, this issue always needs particular attention before considering the application of this type of algorithm in bioprocess control and for this case it will be discussed accordingly in the results and discussion part.

Basic structure of the control algorithm used is the PSD control law in the incremental form with proportional, summing and differencing terms

$$\Delta u(n) = \alpha(n)e(n) + \beta(n)\Delta e(n) + \gamma(n)\Delta^2 e(n)] \qquad (1)$$

where u is the output variable of the controller, Δu is its increment, e, Δe and $\Delta^2 e$ are the control error and its first and second backward differences, i.e. $\Delta u(n) = u(n) - u(n-1)$, $\Delta e(n) = e(n) - e(n-1)$ and $\Delta^2 e(n) = e(n) - 2e(n-1) + e(n-1)$, respectively, and finally α is the overall gain of the controller.[31] The derivation of the adaptive algorithm below will follow the original paper by Maršík.[30, 31]

The adaptation criterion κ**,** which is used for tuning of the controller, represents oscillation rate of the control error signal. Oscillation rate κ is a dimensionless criterion, which is of heuristic character as explained above. It is defined by ratio of the frequency f_e of the error signal transits thru zero value and the frequency f_v of the first derivative of the error signal transits thru zero value.

$$\kappa = \frac{f_e}{f_v} \qquad (2)$$

It expresses the extent of the error oscillations; i.e. the more damped is the process the smaller is the criterion value and conversely the more oscillating is the process the greater is the criterion value. In the case of the optimal response, the error has two damped half-waves only and accordingly $\kappa = 0.5$ because the error has only one zero level transit and its derivative has two zero level transits as depicted in Fig. 2.

Because determination of the frequencies f_e, f_v is difficult especially in case of infrequent disturbances, these frequencies can be calculated indirectly using Rice's formula[38] for calculation of the number of transits of the continuous stochastic centred Gauss signal thru zero value.

$$f_e = \frac{1}{\pi}\sqrt{\frac{\sigma_v^2}{\sigma_e^2}} \quad f_v = \frac{1}{\pi}\sqrt{\frac{\sigma_a^2}{\sigma_v^2}} \tag{3}$$

where σ_e^2 is variance of error, σ_v^2 is variance of its first derivative (velocity v), and σ_a^2 is variance of its second derivative (acceleration a). For discrete signals the derivatives are replaced by differences and formulas for calculation of f_e, f_v will change to

$$f_e(n) = \frac{1}{\pi}\sqrt{\frac{\sum_{i=0}^{n}(\Delta e(i))^2}{\sum_{i=0}^{n} e^2(i)}}$$

$$f_v(n) = \frac{1}{\pi}\sqrt{\frac{\sum_{i=0}^{n}(\Delta^2 e(i))^2}{\sum_{i=0}^{n} \Delta e(i)^2}} \tag{4}$$

in the n-th sample, where $e(i)$ is the error in the i-th sample.

Continuous adaptation requires further rearrangement of the formulas above with respect to the continuous exponential forgetting with an appropriate velocity. Therefore, the variances in the Eq. 4 will be computed by passing of the variables e^2, $(\Delta e)^2$ and $(\Delta^2 e)^2$ through the first order filter and denoted as

$$\overline{e^2(n)} = \frac{e^2(n) + \tau(n)\overline{e^2(n-1)}}{1+\tau(n)} \tag{5}$$

and as well as for $\overline{(\varnothing e(n))^2}$ and $\overline{(\varnothing^2 e(n))^2}$.

The time constant $\tau(n)$ of the first order filter determines the velocity of exponential forgetting and is defined as $\tau(n) = 2 / f_v(n-1)$.

Finally, for the value of the oscillation criterion κ in the *n-th* sample we obtain

$$\kappa(n) = \frac{f_e(n)}{f_v(n)} = \frac{\overline{(\Delta e(n))^2}}{\sqrt{\overline{e^2(n)}\,\overline{(\Delta^2 e(n))^2}}} \tag{6}$$

Because the value of the oscillation rate k depends on overall gain α, it is possible to create an adaptation loop, which will adjust gain α in order to reach approximate optimum of $\kappa = 0.5$. Adaptation loop can be organized as a standard feedback control loop with a summing controller, which will maintain value of the criteria κ on pre-specified value $\kappa_{SP} = 0.5$ by means of the gain α as a manipulated variable. New values of α are then calculated according to the conceptual equation

$$\Delta\alpha = 0.5 f_v \alpha\left(\frac{\kappa_{SP}}{\kappa} - 1\right) \tag{7}$$

where the product 0.5 f_v is of a heuristic nature, which facilitates conformity of the adaptation and control rates. It is impossible to do adaptation faster than control.

Resulting formula for adaptation of the overall gain α is

$$\Delta\alpha(n) = \alpha(n-1)\frac{0.5}{\pi}\sqrt{\frac{\overline{(\Delta^2 e(n))^2}}{\overline{(\Delta e(n))^2}}} \cdot \left(\frac{\kappa_{SP}\sqrt{\overline{e^2(n)}\,\overline{(\Delta^2 e(n))^2}}}{\overline{(\Delta e(n))^2}} - 1\right) \tag{8}$$

The algorithm for the continuous adaptation thus consists of formulas for $\tau(n)$, $\overline{e(n)^2}$, $\overline{(\Delta e(n))^2}$, $\overline{(\Delta^2 e(n))^2}$, and $\Delta\alpha(n)$.

Thus far, the adaptation of the overall gain α has been derived. For the adaptation of the remaining coefficients β and γ another heuristic can be applied. In order to have equally significant contribution to the total controller output, all three terms of the controller should have comparable quantity. Therefore, the β and γ coefficients are adapted in such a way, so that standard deviations of all three terms are the same:

$$\sqrt{\overline{e^2}} = \beta\sqrt{\overline{(\Delta e)^2}} = \gamma\sqrt{\overline{(\Delta^2 e)^2}} \tag{9}$$

From this condition the formulas for adaptation of β and γ parameters are hence simply

$$\beta = \sqrt{\frac{\overline{e^2}}{\overline{(\Delta e)^2}}} \quad \gamma = \sqrt{\frac{\overline{e^2}}{\overline{(\Delta^2 e)^2}}} \tag{10}$$

In the algorithm, it is necessary to handle situations when the error is zero or near to the zero level and hence a division by zero error or inaccuracy in the adaptation calculation could occur. Accordingly, if the error decreases below a certain level (e.g. 10 % of the standard deviation), calculations for the adaptation are stopped – if there is no need to control then there is no need to adapt.

Results and discussion

Maršík's controller implementation for fed-batch bioprocess control

Application of the Maršík's adaptive algorithm to the control of the *Saccharomyces cerevisiae* D7 yeast fed-batch cultivations necessitated the enhancement of the controller with additional features.

1. It is preferable to have an option for a modification of the condition for turning off the adaptation especially during testing of the controller. So the fixed 10 % level in this condition has been replaced by a variable parameter φ

$$e(n)^2 \le \sigma_e^2(n-1)\frac{\phi}{100} \tag{11}$$

which denotes the percentage of the error variance and can be changed by the operator through the cultivation. The error variance is calculated according to the original paper[30] using a filter with slow forgetting relatively to the calculations of the variances in Eq. 5.

$$\sigma_e^2(n) = \frac{e^2(n) + 3\tau(n)\sigma_e^2(n-1)}{1+3\tau(n)} \tag{12}$$

2. For practical usage of the controller, it is also important to turn off adaptation if saturation of the actuator occurs. The adaptation is undesirable as long as the actuator is saturated at the limits of its range.

3. For the removal of an eventual high frequency noise from the control error signal a filtration was added and it was realized by means of the formula

$$e^*(n) = e^*(n-1) + c\big(e(n) - e^*(n-1)\big) \tag{13}$$

The terms e and e^* are the error and the filtered error respectively. Turning this filtration off and on and setting up also the level of the filtration can be accomplished by adjusting the constant c of the filter in the interval $\langle 0;1\rangle$.

Finally, the important issue of stability had to be addressed because fed-batch operated bioprocesses are known to be inherently unstable.[39] Despite the fact that this inherent instability appears to have limited impact on the way many industrial fed-batch fermentations are still operated – application of open-loop control strategies in the form of feeding recipes (i.e. repeating feeding profiles from successful fermentations) is still widespread[40] – in the presented case this issue had to be examined because the original design of the Maršík's adaptive algorithm is based on the assumption of inherent process stability. Specifically in the case of *Saccharomyces cerevisiae* fed-batch fermentations, for which a non-monotonic Haldane-type biomass growth kinetics is characteristic, it has been shown that process set points corresponding to the left flank of the Haldane kinetics, i.e. set points related to process states with no carbon-substrate inhibition, low ethanol concentration and specific biomass growth rates lower than the maximum specific biomass growth rate, are stable.[41] For the process in question, i.e. *Saccharomyces cerevisiae* D7 fed-batch fermentation for ergosterol production, this additional condition is fulfilled as the process is operated exclusively within this stable region since lower specific growth rates are also favourable for the product formation[37] and hence under these conditions the proposed adaptive control strategy is applicable.

Experiments

Two experiments were carried out in the laboratory bioreactor for verifying functionality of the adaptive controller itself and within the whole scheme of the knowledge-based control system (Fig. 1). A further aim of the experiments was to find out the influence of the parameters that affect the adaptation process. The adaptive controller used the carbon dioxide concentration in the exhaust gas as the controlled variable and the feed rate of glucose as the manipulated one. Hence the adaptive controller was responsible for standard regulatory control (that is maintaining CO_2 values near or equal to CO_{2SP}) in varying process conditions caused both by external disturbances and variations in the microbial culture itself. The CO_2 set point profile for the controller was computed continuously by the supervisory knowledge-based system using the mechanism for on-line set point adjustments as described and published previously in the paper by Hrnčiřík et al.[36] However, the primary aim of the experiments was the testing of the controller under process conditions wider than those of normal operation favourable for ergosterol production.[37] In order to achieve this, the settings of the supervisory knowledge-based system were set in such a way as to allow CO_2 set point adjustments leading the process towards and slightly beyond the upper limits of normal operation into process regions characterized

Table 1 – *Changes in $CO2_{SP}$ slope values during Cultivation I together with the values of the current metabolic state and related process variables*

Time (h)	$CO2_{SP}$ (vol %)	$CO2_{SP}$ slope (vol % h^{-1})	Metabolic state	EtOH (wt %)	dEtOH/dt (wt % h^{-1})	RQ
1.57	0.55	0.00	3	0.00	~ 0	1.23
2.17	0.55	0.10	3	0.00	~ 0	1.15
2.87	0.62	0.20	3	0.01	> 0	1.15
3.57	0.76	0.15	3	0.04	> 0	1.29
4.78	0.94	0.10	3	0.11	> 0	1.37
5.68	1.03	0.05	3	0.16	> 0	1.32
6.85	1.09	0.00	3	0.23	> 0	1.25
7.95	1.09	0.05	2	0.26	> 0	1.14
9.58	1.17	0.07	2	0.31	~ 0	1.09
15.02	1.54	0.10	2	0.36	~ 0	1.06

by mixed oxidative-fermentative metabolism with RQ values above 1.3 and moderate ethanol production – metabolic state 3. Under normal operation, the process is maintained at the border between purely oxidative and mixed oxidative-fermentative metabolism with glucose as substrate – metabolic state 2 (for the definition of all metabolic states see Hrnčiřík et al.[36]).

Cultivation I

In the first experiment, the coefficients of the controller were preset to the values of 1. These values were verified in preliminary experiments as sufficiently low to guarantee stable control from the start of the controller. The initial value of $\sigma_e^2(0)$ was set to the rough estimate of real error variance and the initial values of $\overline{e^2(0)}$, $\overline{(\Delta e(0))^2}$ and $\overline{(\Delta^2 e(0))^2}$ were set equal to $0.1\sigma_e^2(0)$, just to prevent division by zero, their influence will however gradually disappear due to the exponential forgetting. The control period was set to 1 minute to be slightly longer than the system transportation lag. The value of the parameter φ in Eq. 11 for turning off the adaptation when the error variance is small had been set to the value of five. The filtration of the control error was turned off.

The feed rate was adjusted manually during the first one and a half hour of the experiment to increase the CO_2 concentration in the exhaust gas in order to reach reasonably high set point of the CO_2 concentration for the controller. After that, the controller was switched on with the initial set point slope calculated by the supervisory level. In the starting phase of the cultivation, the set point was gradually set by the supervisory level to values, which forced the culture into the metabolic state 3 (mixed oxidative-fermentative metabolism with glucose as substrate) with RQ values above 1.3. This state lasted until approx. the 8th hour of the experiment, when the metabolic state 2 (border between purely oxidative and mixed oxidative-fermentative metabolism with glucose as substrate) was set, which was maintained till the end of the cultivation. The entire course of the CO_2 set point profile, as defined by individual set point slope values set by the supervisory level presented in Table 1, is shown in Fig. 3 together with CO_2 concentration and feed rate. In Figs. 4 and 5 the courses of the ethanol concentration, biomass concentration

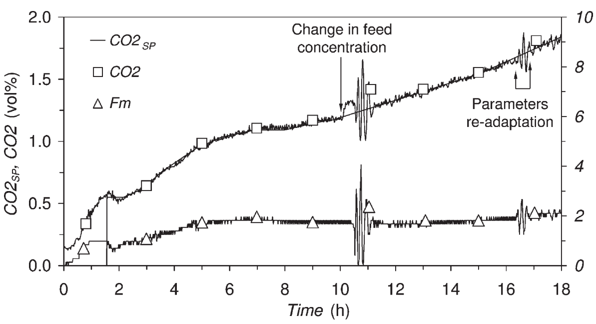

Fig. 3 – *Time courses of selected process variables during Cultivation I: CO2 – off gas carbon dioxide concentration, $CO2_{SP}$ – off gas carbon dioxide concentration set point and Fm – feed rate*

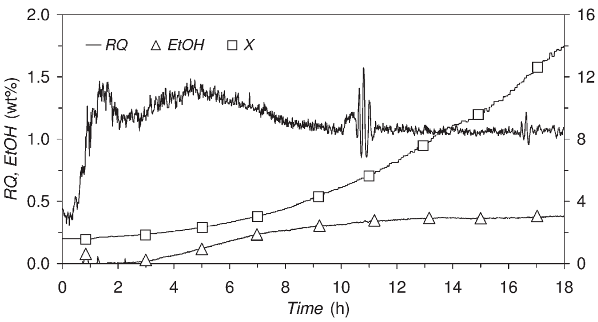

Fig. 4 – *Time courses of selected process variables during Cultivation I: RQ – respiratory quotient, EtOH – ethanol concentration and X – biomass (dry cell weight) concentration*

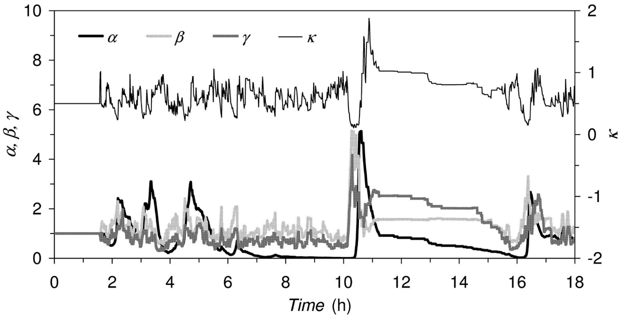

Fig. 5 – *Time courses of the oscillation rate κ and controller parameters α, β and γ during cultivation I*

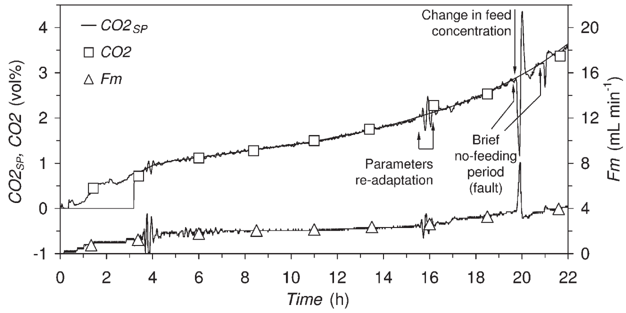

Fig. 6 – *Time courses of selected process variables during Cultivation II: CO2 – off gas carbon dioxide concentration, $CO2_{SP}$ – off gas carbon dioxide concentration set point and Fm – feed rate*

with respiratory quotient and of the controller parameters with k criterion are presented respectively.

The controller performed well during this cultivation with standard deviation in smooth phases on the level of 0.02 vol %. In the 10^{th} hour, a disturbance has been introduced intentionally by using a new more concentrated feeding medium (135 g L^{-1} in place of 125 g L^{-1}). This disturbance was eliminated by the controller within less than an hour exhibiting oscillations with absolute error above 0.2 vol %. Other significant oscillations in the 16^{th} hour were caused by a rapid re-adaptation of the parameters as a result of the process shifting towards a higher CO_2 production induced by set point slope changes. In both cases the disturbance meant that the control error was no longer oscillating around the zero value and hence the κ coefficient decreased below its set point of 0.5. The controller consequently steeply increased the gain to elevate κ and also appropriately changed the other two coefficients thus facilitating good regulatory process in this situation. Subsequently the parameters were adapted to lower values again according to κ coefficient. In this manner, the Maršík's controller readapts its parameters to follow the value of κ_{SP} ordinarily.

Throughout this experiment the specific biomass growth rate did not exceed 0.20 h^{-1}, final yeast biomass (dry cell weight) concentration was 13.8 g L^{-1}, the attained concentration of sterols in yeast biomass dry matter was 1.34 wt % and the concentration of ergosterol in total sterol fraction was 88 wt %.

Cultivation II

In the Cultivation II, the coefficients of the controller had been preset as before but the filtration of the error was turned on, with the filter constant *c* set to 0.7. The cultivation was initiated in the same manner as the Cultivation I, first the feed rate had been adjusted manually and then the controller was switched on in the 3^{rd} hour. Then, in the first half of the cultivation, the set point was, as in the Cultivation I, gradually set by the supervisory level to values, which forced the culture into the metabolic state 3 (mixed oxidative-fermentative metabolism with glucose as substrate) with RQ values above 1.3. During the second half of the cultivation, from the 11^{th} hour approximately, the metabolic state 2 (border between purely oxidative and mixed oxidative-fermentative metabolism with glucose as substrate) was maintained up to the 20^{th} hour, when the dissolved oxygen tension decreased below 15 sat % causing an oxygen limitation and leading to a moderate increase in the ethanol concentration during the final two hours of the experiment (see Fig. 7). The onset of oxygen limitation was caused by biomass reaching concentrations above 20 g L^{-1} and hence surpassing the current aeration capacity of the bioreactor. During this period of the oxygen limitation, the set point of CO_2 was adjusted thus to retain the exponential trend in the CO_2 concentration as before. Changes in the slope are listed in Table 2.

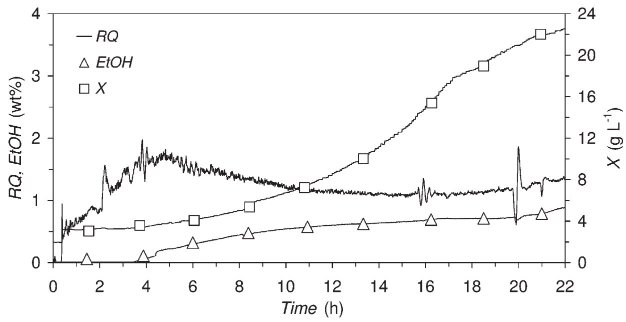

Fig. 7 – *Time courses of selected process variables during Cultivation II: RQ – respiratory quotient, EtOH – ethanol concentration and X – biomass (dry cell weight) concentration*

In the 8^{th} hour of the cultivation, the length of the control period was changed by the operator from 1 to 2 minutes, because of greater oscillations than in the Cultivation I. The increase of oscilla-

Table 2 – *Changes in $CO2_{SP}$ slope values during Cultivation II together with the values of the current metabolic state and related process variables*

Time (h)	$CO2_{SP}$ (vol %)	$CO2_{SP}$ slope (vol % h^{-1})	Metabolic state	EtOH (wt %)	dEtOH/dt (wt % h^{-1})	RQ	DO (sat %)
3.18	0.75	0.25	2	0.01	~ 0	1.51	76
4.20	0.97	0.12	3	0.09	> 0	1.63	75
5.00	1.06	0.07	3	0.22	> 0	1.66	77
10.80	1.47	0.38	3	0.56	> 0	1.16	57
12.83	1.67	0.10	2	0.62	~ 0	1.11	48
14.15	1.83	0.12	2	0.64	~ 0	1.10	42
15.02	1.95	0.14	2	0.66	~ 0	1.09	38
16.88	2.25	0.16	2	0.71	~ 0	1.06	28
17.18	2.30	0.18	2	0.71	~ 0	1.11	26
18.03	2.47	0.20	2	0.71	~ 0	1.09	22
18.35	2.54	0.22	2	0.71	~ 0	1.13	20
19.00	2.70	0.24	2	0.71	~ 0	1.14	18
19.50	2.83	0.26	2	0.72	~ 0	1.20	16
20.23	3.03	0.28	3	0.75	> 0	1.20	9
20.52	3.12	0.30	3	0.76	> 0	1.23	8
20.72	3.18	0.32	3	0.77	> 0	1.27	8
21.00	3.28	0.34	3	0.79	> 0	1.12	8
21.33	3.40	0.36	3	0.82	> 0	1.33	6

tions occurred due to the increase of transportation lag of the system caused by the turning on of the control error filtration, meaning as a result that the lag value was greater than control period of the control algorithm itself. In order to eliminate this problem the control period was set to 2 minutes. In the 15th hour the value of the filter constant was changed from 0.7 to 0.3 to check the effect of this value on the controller performance. The controller performance consequently improved and adaptation of the parameters was less frequent thereafter.

The second significant oscillation in this experiment (the first one followed the switch-on of the controller) in approx. the 16th hour with maximal absolute error of 0.3 vol % was induced by a regular rapid re-adaptation of the controller parameters in the case of changes of the controlled process. The reason was an enforcement of the process to a high CO_2 production by set point slope, probably in conjunction with a prior sample of the volume equal to approx. 10 % of the bioreactor broth that reduced the number of cells producing CO_2.

As an intentional disturbance, the substrate concentration in the feeding medium was increased from 125 g L^{-1} to 156 g L^{-1} after the 20th hour of the cultivation. However, it was accompanied by unwanted clogging of the output piping from the feeding pump, which occurred just before the change in the feeding solution concentration. Due to this failure, the CO_2 concentration decreased, i.e. it changed in the opposite direction than anticipated originally. Nevertheless, the controller was able to suppress the influence of both disturbances within one hour. A less distinct fall in CO_2 concentration in the 21st hour was caused by a short break in the feeding due to disconnection of the supply tube from the reservoir, the corresponding disturbance was however subsequently effectively eliminated by the controller. See Figs. 6 to 8 for the results of the Cultivation II.

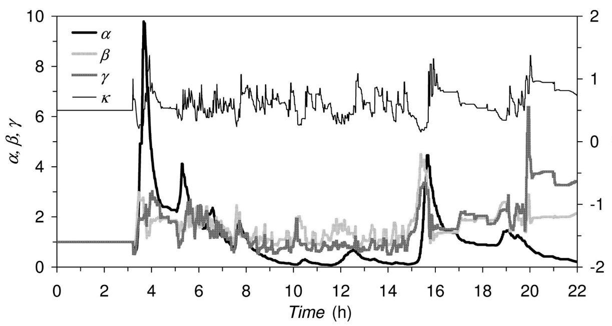

Fig. 8 – *Time courses of the oscillation rate k and controller parameters a, b and g during cultivation II*

Throughout this experiment the specific biomass growth rate did not exceed 0.11 h^{-1}, final yeast biomass (dry cell weight) concentration was 23.4 g L^{-1}, the attained concentration of sterols in yeast biomass dry matter was 1.77 wt % and the concentration of ergosterol in total sterol fraction was 85 wt %.

In both experiments the choice of the set point profile was done by the supervisory knowledge-based level ensuring that the yeast culture was maintained in metabolic states with no carbon-substrate inhibition and hence low ethanol concentration, therefore the adaptive controller was operating within a region of stable set points on the left flank of the non-monotonic kinetics.[41]

Conclusion

An adaptive strategy for the control of the *Saccharomyces cerevisiae* D7 fed-batch cultivation process capable of coping with process uncertainties and dynamical changes of process properties was introduced and studied. The CO2MAR adaptive controller is based on the Maršík's algorithm for direct adaptation of the discrete PID controller parameters and it was implemented in the BIOGENES II control system, which was used for experiments.

Two experimental fed-batch cultivations were carried out, which have verified good CO2MAR controller functionality in the changing environment. Particularly, the controller was maintaining the carbon dioxide concentration in the exhaust gas on the set point profile, which slopes were determined by the supervisory knowledge-based level. The response of the controller to the disturbances in the feeding (substrate concentration changes in the feeding medium, faults of the feeding pump) proved to be very satisfactory as was its performance throughout the whole cultivation, during which the biomass concentration increased several times with corresponding changes in the dynamics of the yeast's culture.

Even though the experimental tests were not primarily aimed at maximising the ergosterol production, the obtained results are fully comparable with those for the same strain reported by Rychtera et al.[37]

CO2MAR controller used in connection with the supervisory knowledge-based level represents a robust and flexible tool for control of biotechnological processes, which are all inherently non-stationary. In addition, it operates on the basis of information available in the on-line measured process data only with no mathematical model. It can be used not only for control of the presented process but also for a number of similar processes using microorganisms with the same type of metabolism, e.g. genetically modified *Saccharomyces cerevisiae* yeast, and operating under similar process conditions.

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List of symbols and abbreviations

α – overall gain of the controller
β – proportional coefficient of the controller
γ – difference coefficient of the controller
Δ – first backward difference
Δ^2 – second backward difference
φ – fraction of error variance, %
κ – oscillation rate
κ_{SP} – oscillation rate set point
π – pi
v – velocity
a – acceleration
σ^2_e – error variance
σ^2_v – error's first derivative variance
σ^2_a – error's second derivative variance
τ – time constant of the first order filter
c – constant for set point filtration
e – error
e^* – filtered error
$\overline{e^2}$ – σ^2_e computed by passing of the variable e^2 through the first order filter
$\overline{(\Delta e)^2}$ – σ^2_v computed by passing of the variable $(\Delta e)^2$ through the first order filter
$\overline{(\Delta^2 e)^2}$ – σ^2_a computed by passing of the variable $(\Delta^2 e)^2$ through the first order filter
f_e – frequency of the zero level transits of the error
f_v – frequency of the zero level transits of the first derivative of the error
i – summation index
n – number of sample
u – controller output
CO_2 – carbon dioxide
CO2 – off-gas carbon dioxide concentration, vol %
$CO2_{SP}$ – set point of CO2 for the controller, vol %
CO2MAR – implementation of Maršík's adaptive controller to CO2 control
dEtOH/dt– ethanol concentration trend, wt % h^{-1}
DO – dissolved oxygen tension, sat %
EtOH – ethanol concentration, wt %
Fm – glucose feed rate, mL min^{-1}
OLE – Object Linking and Embedding
PC – personal computer
PID – proportional-integral-derivative (controller)
RQ – respiratory quotient
X – biomass (dry cell weight) concentration, g L^{-1}

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