Software Sensors for Biomass Concentration Estimation in Filamentous Microorganism Cultivation Process

P. Hrnčiřík,[a,*] T. Moucha,[b] J. Mareš,[a] J. Náhlík,[a] and D. Janáčová[c]

[a]Department of Computing and Control Engineering, University of Chemistry and Technology, Prague, Technická 5, 166 28 Praha 6, Czech Republic

[b]Department of Chemical Engineering, University of Chemistry and Technology, Prague, Technická 5, 166 28 Praha 6, Czech Republic

[c]Faculty of Applied Informatics, Tomas Bata University in Zlín, Nad Stráněmi 4511, 760 05 Zlín, Czech Republic

doi: 10.15255/CABEQ.2018.1387

Original scientific paper
Received: May 23, 2018
Accepted: March 4, 2019

In this study, the potential of two software sensors for on-line estimation of biomass concentration during cultivation of filamentous microorganisms is examined. The first sensor is based on common bioreactor off-gas analyses, and uses the assumption of the biomass concentration linear dependence on the square root of cumulative O_2 consumption. Parameters of the semi-empirical data-driven software sensor based on off-gas analysis were calculated from experimental cultivation data using linear regression. The second sensor is based on biocalorimetry, *i.e.,* the on-line calculation of metabolic heat flux from general enthalpy balance of the bioreactor. The software sensor based on biocalorimetry thus essentially represents a model-driven approach, making use of a fundamental process model based on the enthalpy balance around the bioreactor. This approach has been combined with the experimental identification of the specific biomass heat production, which represents the main process-specific parameter of the software sensor based on biocalorimetry. For this sensor, the accuracy requirements on the process variable on-line measurements were also analysed. The experimental data from the pilot-scale antibiotics Nystatin production by a bacterium *Streptomyces noursei* were used to calculate the specific bioprocess heat production value using linear regression. The achieved results enabled us to propose a new on-line indicator calculated as the ratio of the outputs of both sensors, which can serve as a timely warning of the risk of undesired nutritional conditions of a culture characterized as underfeeding.

Keywords:
software sensors, filamentous fermentation, biomass, calorimetry, process monitoring

Introduction

The software sensor ("soft sensor" or "software sensor") already represents an established concept in the field of production process monitoring. The term "software" indicates the fact that the output signal is largely the result of more or less complex calculations realized in a program module. The term "sensor" then means that the entire software sensor provides on-line information about the monitored process, similarly to traditional hardware sensors[1]. The basic principle of software sensors is the use of one or more relatively easy on-line measurable process variables to estimate other variables or process indicators that are difficult to measure in on-line mode, or can be measured with too long sampling periods. In principle, it is possible to distinguish between two basic types of software sensors[2,3].

– "grey-box" sensor, also referred to as "model-driven" – based on a mathematical model of a process based on physical, chemical or biological relationships with experimental identification of unknown parameters from historical process data;

– "black-box" sensor, also referred to as "data-driven" – is used in cases where a mathematical model of the relation between inputs and outputs of the software sensor is not known. Therefore, the mathematical description of this relation is derived from historical process data using appropriate computational tools (regression analysis, neural networks, etc.).

Software sensors based on mathematical models that are used in chemical and biotechnological

*Corresponding author:
fax: +420 220 445 053 and e-mail: Pavel.Hrncirik@vscht.cz

processes typically result from mass or enthalpy balances supplemented, for example, with kinetic equations. In addition to the choice of a suitable calculation method, the key factor for the functionality of the sensor is also the choice of suitable input, on-line measured quantities. In the case of biotechnological processes, software sensors that use on-line measurement of the composition of the bioreactor off-gases are quite common[4]. These on-line measurements are successfully used for the on-line calculation of so-called derived quantities, such as the oxygen uptake rate (OUR), the carbon dioxide production rate (CPR), the respiratory quotient (RQ), or the volumetric mass-transfer coefficient in the bioreactor (k_La). More sophisticated software sensors are able to estimate the key indicators of bioprocesses, such as the biomass concentration and the biomass growth rate based on the abovementioned online measurements (often combined with other on-line measurable process variables, such as pH, temperature, or dissolved oxygen concentration) of the concentration and production rates of main products[5–9].

The interest in the application of software sensors in the monitoring of production bioprocesses increases proportionally to the increasing demands on the quality of the production process itself and the resulting products. In particular, this is the case in the field of pharmaceutical products, as a result of the trends called "Quality by Design" and "Process Analytical Technology – PAT". Compared to costly and relatively complex analytical technologies, the application of software sensors is often a more convenient solution for monitoring especially those bioprocesses that are operated in the form of fed-batch cultivations, for which complex process dynamics, considerable variability due to the variable input composition, and frequent change of the production bioprocesses due to the production of various products, are typical. In these cases, software sensors are successfully used not only to monitor the production process itself, but also to evaluate the quality of input raw materials or the quality of production microbial cultures at the very beginning of the production process used as a seed culture[10–12].

In this paper, two alternative approaches to the development of software sensors for on-line estimation of biomass concentration of filamentous microorganisms will be proposed. The first approach is based on the on-line measurement of the composition of the bioreactor off-gases, specifically on the close relationship between the cumulative values of oxygen consumption or carbon dioxide production, and the biomass concentration in the bioreactor. The second approach is based on biocalorimetry, *i.e.,* the on-line calculation of metabolic heat flux from the general enthalpy balance of the bioreactor. The process used in this study is the industrial production of antibiotics Nystatin, which is a polyene antifungal medication that is produced as a secondary product from the bacterium *Streptomyces noursei*.

Materials and methods

Process description

The production of the antibiotics Nystatin is carried out in large bioreactors with the volume of 50,000 L. For the purposes of pilot-scale testing, the production process can also be carried out in smaller bioreactors with the volume of 300 L. The production process can be divided into two distinct phases. In the initial phase, the main objective is the maximisation of the cellular growth. In the subsequent second phase, the microbial culture produces the secondary product Nystatin. The bioreactors are equipped with a set of sensors providing online measurements of most of the important process variables influencing the product development (*e.g.,* pH, dissolved oxygen concentration, temperature, pressure, etc.). Still, it is necessary to take samples regularly for laboratory analyses for variables that are not measured on-line (*e.g.,* biomass, nutrient, and product (Nystatin) concentrations, viscosity of medium, etc.). Specifically in the initial phase, precise and timely information related to cell growth is important for efficient process monitoring, and hence the application of software sensors for on-line estimation of biomass concentration presents a cost-efficient solution that can improve the quality of the Nystatin production process substantially. Due to preserving manufacturing secrets, the production process cannot be described in more detail, and therefore in all charts published in this paper, unit-scale representations are used for all data sets.

Software sensors based on off-gas analysis

Software sensors for biomass concentration estimation based on off-gas analysis data have been reported in a number of applications[13–15]. The rates of oxygen consumption and carbon dioxide production are known to be closely related to the biomass growth in microbial cultivation processes. Specifically, in aerobic microbial cultivation processes, this relationship can be described by the Luedeking-Piret-type of equation[13] (Eq. 1 for OUR and Eq. 2 for CPR, respectively).

$$OUR = Y_{O/X} \cdot \frac{\mathrm{d}c_X}{\mathrm{d}t} + m_O \cdot c_X \tag{1}$$

$$CPR = Y_{C/X} \cdot \frac{\mathrm{d}c_X}{\mathrm{d}t} + m_C \cdot c_X \tag{2}$$

where $Y_{O/X}$ (kg oxygen consumed per 1 kg biomass produced) is the yield coefficient relating oxygen consumption to biomass production, c_X (kg m^{-3}) is the microbial cell concentration in the bioreactor, t (min) is the cultivation time, m_O (kg oxygen consumed per 1 kg biomass per 1 min) is the oxygen consumption coefficient related to maintenance, $Y_{C/X}$ (kg carbon dioxide produced per 1 kg biomass produced) is the yield coefficient relating carbon dioxide production to biomass production, and m_C (kg carbon dioxide produced per 1 kg biomass per 1 min) is the carbon dioxide coefficient related to maintenance.

If the yield coefficients can be assumed to be constant, and the contribution of the maintenance part of equations 1 and 2 is low enough to be neglected, then the relationship between the cumulative (integral) values of O_2 consumption (COC) and CO_2 production (CCP), and the biomass concentration can even have the form of linear dependence[13,16]. However, in the specific case of filamentous microorganisms with complex morphology, a linear correlation was observed between biomass concentration and the square root of the cumulative CO_2 production[16]. This linear relationship between the biomass concentration and the square root of the cumulative CO_2 production must be considered as semi-empirical, as it has no immediate theoretical explanation in terms of the underlying microbial growth kinetics[16].

Therefore, in this study, it was decided to explore four different types of biomass concentration software sensors based on off-gas analysis data.

Type 1: biomass concentration is assumed to be linearly dependent on cumulative O_2 consumption;

Type 2: biomass concentration is assumed to be linearly dependent on cumulative CO_2 production;

Type 3: biomass concentration is assumed to be linearly dependent on the square root of cumulative O_2 consumption;

Type 4: biomass concentration is assumed to be linearly dependent on the square root of cumulative CO_2 production.

Software sensors based on calorimetry

The literature survey says that the bioprocess heat production can be determined by the enthalpy balance of a bioreactor[17–19]. Establishing the quantitative connection between the bioprocess heat production and the biomass concentration, we can obtain the tool for online monitoring of the biomass growth.

For this purpose, the process variables depicted in the fermentation scheme (Fig. 1) need to be measured. The measured process variables can then be used in the following heat balance (Eq. 3).

The process variables usually measured in practice are used in the balance, for example, the volumetric flow multiplied by density instead of the mass flow. In the equation, the first and second term of the right-hand side correspond with the sensible heats of cooling water and aeration gas, and contribute to the total enthalpy flow through the apparatus by –90 % and –3 %, respectively (the minus sign represents heat losses, the plus sign represents heat gains). The term described as the sum of "*k.additive*" represents the enthalpy brought into the fermentation broth by the addition of nutrients, buffers, etc. Further terms after the sum of "*k.additive*" represent the following quantities (and contribute to the total enthalpy flow in the following amounts): heat loss/gain through the vessel wall (±1 %), heat losses due to water evaporation into the aeration gas (–4 %), and CO_2 desorption (–3 %), respectively. Then the heat production terms follow, namely the amount of heat generated by the microbial culture biomass Q_{BIO} (+85 %), followed by heat production due to stirring enthalpy dissipation P_{imp} (+15 %), and gas bubbles expansion (+0.5 %), respectively. The percentage values stated above in brackets for individual equation terms are estimates published in literature[17,18]. The value of Q_{BIO} can be calculated on-line from the heat balance (Eq. 3) when all other process variables are measured on-line. The percentage values show that the major part of the heat flow originates from the bioprocess itself (around 85 %), while the secondary heat sources (*e.g.,* impeller power input or gas bubbles expansion) ac-

$$\begin{aligned}
&\left\{c_P \cdot \rho \cdot V\right\}_L \frac{\mathrm{d}T_L}{\mathrm{d}\tau} = \left\{c_P \cdot \rho \cdot \dot{V} \cdot \left(T_{in} - T_{out}\right)\right\}_w + \left\{c_P \cdot \rho \cdot \dot{V}_{in} \cdot T_{in}\right\}_{Air} - \left\{c_P \cdot \rho \cdot \dot{V}_{out} \cdot T_{out}\right\}_{Air} + \\
&+ \sum_{k.additive} F_k \cdot c_{P,k} \cdot \rho_k \cdot \dot{V}_k \cdot \left(T_{in}\right)_k - \propto_{Air} \left(T_{surf} - T_{ambient}\right) \cdot A_{surf} - \\
&- \left\{\frac{1}{RT_{out}} \dot{V}_{out} \cdot pw^0\left(T_L\right) - \frac{1}{RT_{in}} \dot{V}_{in} \cdot pw_{in}\right\}_{Air} \cdot \Delta H_{evap,w}\left(T_L\right) - \\
&- \frac{1}{RT_{out,Air}} \dot{V}_{out,Air} \cdot p\mathrm{CO}_2 \cdot \Delta H_{des,\mathrm{CO}_2} + Q_{BIO} + P_{imp} + \Delta h \cdot \rho_L \cdot g \cdot \dot{V}_{in,Air}
\end{aligned} \tag{3}$$

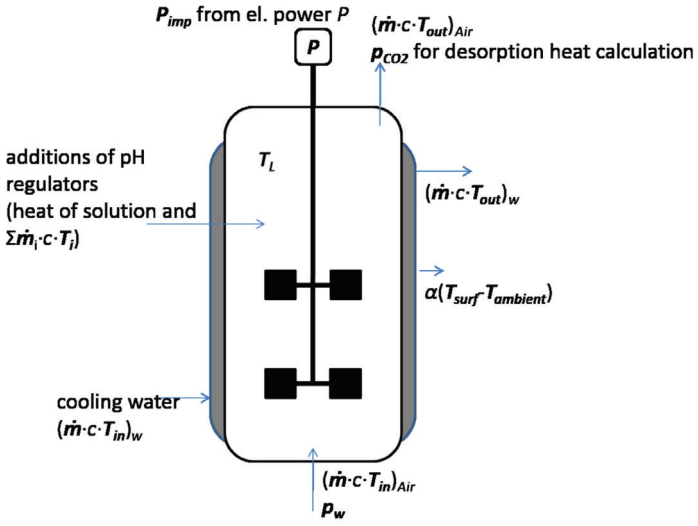


Fig. 1 – *Bioreactor scheme with the process variables (in Italics and Bold) which are needed to be measured in calorimetry*

count for only a minor part. The fraction of the heat produced by the bioprocess is higher in large-scale fermenters, where the higher volume-to-surface ratio occurs, so the calorimetric determination of biomass content will generally be more accurate in larger apparatuses.

Analytical methods and process data measurement

Off-gas composition measurements

The oxygen and carbon dioxide concentrations in the off-gas from the bioreactor were measured by Servomex Servopro process analysers. The resulting off-gas composition measurement data were then used for on-line calculation of O_2 uptake (OUR) and CO_2 production (CPR) rates, as well as their corresponding cumulative values – cumulative O_2 consumption (COC) and cumulative CO_2 production (CCP), using Eqs. (4–7):

where $\dot{V}_{\text{in},Air}$ ($m^3\ s^{-1}$) is the volumetric air flow rate at the inlet to the bioreactor, V_L (m^3) is the broth volume in the bioreactor, ρ_{Air} (kg m^{-3}) is the air density, M_{Air} (kg mol^{-1}) is the molecular weight of air, ΔO_2 (% vol.) is the difference between oxygen concentrations in the inlet air and the off-gas, ΔCO_2 (% vol.) is the difference between carbon dioxide concentrations in the inlet air and the off-gas, O_2 (% vol.) is the oxygen concentration in the off-gas, CO_2 (% vol.) is the carbon dioxide concentration in the off-gas, N_2 (% vol.) is the nitrogen concentration in the air (assumed to be constant at 79.07 %), M_{O_2} (kg mol^{-1}) is the molecular weight of oxygen, M_{CO_2} (kg mol^{-1}) is the molecular weight of carbon dioxide, k_{conv} is the coefficient for the conversion of concentration values from volume percent into dimensionless volume fraction (k_{conv} = 1/100 = 0.01), t (min) is the current cultivation time, and τ (min) is the variable of integration (takes on values from time 0 to the current t).

Calorimetry measurements

To be able to use the Q_{BIO} parameter, described in Materials and methods (subsection Software sensors based on calorimetry), for the determination of biomass concentration, the specific bioprocess heat production, in W per kg of dry biomass, was determined using a laboratory calorimeter. The calorimeter was filled by 120 mL of fermentation broth. During the pilot plant fed-batch fermentation process, the samples of fermentation broth were taken periodically to measure the unit biomass heat production in the laboratory calorimeter. The broth samples were kept in the calorimeter, where the gas-liquid oxygen transfer sufficient for biomass respiration was ensured by the sufficient value of the volumetric gas liquid interfacial area (sufficient surface area of the broth level). An increase in temperature was recorded for 30 minutes, as shown in Fig. 2, and the specific bioprocess heat production

$$\mathrm{OUR} = \frac{\dot{V}_{in,Air}}{V_L}\cdot\frac{\rho_{Air}}{M_{Air}}\cdot\left(\Delta O_2 - O_2\times\left(\frac{N_2}{N_2+\Delta O_2-\Delta CO_2}-1\right)\right)\times M_{O_2}\cdot k_{conv} \tag{4}$$

$$\mathrm{CPR} = \frac{\dot{V}_{in,Air}}{V_L}\cdot\frac{\rho_{Air}}{M_{Air}}\cdot\left(\Delta CO_2 - CO_2\times\left(\frac{N_2}{N_2+\Delta O_2-\Delta CO_2}-1\right)\right)\times M_{CO_2}\cdot k_{conv} \tag{5}$$

$$\mathrm{COC} = \int_0^t \mathrm{OUR}(\tau)\,\mathrm{d}\tau \tag{6}$$

$$\mathrm{CCP} = \int_0^t \mathrm{CPR}(\tau)\,\mathrm{d}\tau \tag{7}$$

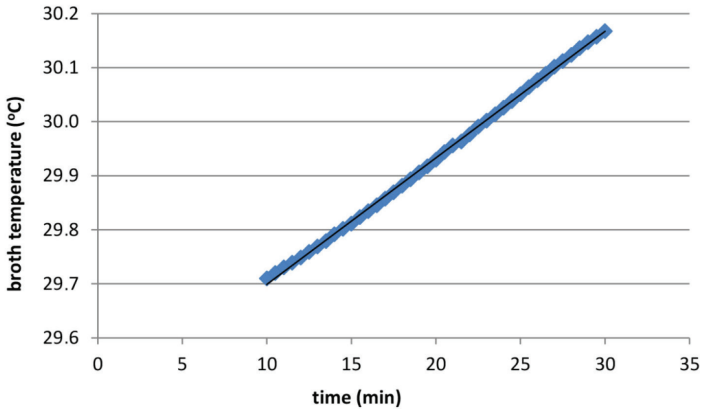


Fig. 2 – *Example of the temperature-time profile measured in the laboratory calorimeter, from the slope of which the bioprocess heat production rate can be determined*

was calculated from the slope of the temperature-time profile as follows:

$$q_{sbio} = \frac{Q_{CAL}}{C_{DB} \cdot V_{CAL}} = c_p \cdot \frac{\mathrm{d}T}{\mathrm{d}\tau} \tag{8}$$

The calorimetry measurements were carried out in the Dewar vessel; therefore, the heat loss through the vessel could be neglected. The impeller used in the calorimeter to support sufficient gas-liquid interfacial transfer was of the diameter equal to 3 cm only, so its power input was much lower than the value of 15 % considered typically for large fermenters, as mentioned in the explanatory text to Eq. 3. From the impeller power number and stirring speeds used in the calorimeter, we calculated its value to be 0.06 W kg^{-1} for 300 rpm, which represents approximately 3 % of the q_{sbio} value, which is compensated by the amount of heat consumed due to the CO_2 desorption (–3 % as mentioned in the explanatory text to Eq. 3). Therefore, by neglecting these two terms, the q_{sbio} data are not distorted significantly.

Overview of the cultivations and laboratory assays

Three pilot-scale Nystatin production cultivations were carried out in the 300-L bioreactor as part of the study of software sensors for biomass concentration estimation. In all three cultivations (C1–3), the off-gas composition was measured on-line by process analysers. In one cultivation (C1), calorimetry measurements were carried out following the procedure described in Materials and methods (subsection Calorimetry measurements).

Biomass concentration in the bioreactor was determined off-line gravimetrically as cell dry mass. The concentration of nutrients (carbohydrate substrates) in the bioreactor was measured as reducing substances using a standard laboratory assay used in the Nystatin production plant.

Results and discussion

Biomass concentration estimation using software sensor based on off-gas analysis

In order to compare the four proposed variants of software sensors based on off-gas composition analysis, a series of three pilot-scale cultivations was carried out, which in its course corresponded to the normal course of production cultivation (*i.e.,* starting with the growth phase of biomass followed by the production stage, where the growth of biomass is already relatively low). From the measured data (Fig. 3), it is obvious that the assumption of the linear relationship between the estimated off-line variable (biomass concentration) and the corresponding considered on-line variable (COC, CCP, sqrt(COC), sqrt(CCP)), is only valid in the case of types 3 and 4 (sqrt(COC), sqrt(CCP)), with slightly better results for type 3 ($R^2 = 0.87$ for sqrt(COC) vs $R^2 = 0.85$ for sqrt(CCP)).

Thus, the resulting software sensor is based on type 3. The software sensor consists of four equations (Eqs. 4, 6, 9, and 10) that allow the on-line estimation of dry biomass concentration in the bioreactor on the basis of the instantaneous rate of oxygen consumption and the initial dry biomass concentration in the reactor after inoculation (Fig. 4). Even though the software sensor is essentially data-driven, no specific data preconditioning methods are needed, because it uses a cumulative process variable (COC) as its input, and therefore, the measurement noise is eliminated as a result of the integration (Eq. 6) involved in calculation of the cumulative process variable (COC).

$$\Delta c_{BIO\_s1}(t) = k1 \cdot \sqrt{\mathrm{COC}(t)} + k2 \tag{9}$$

$$c_{BIO\_s1}(t) = \Delta c_{BIO\_s1}(t) + c_{BIO\_s1}(0) \tag{10}$$

The software sensor was calibrated on the basis of experimental data using linear regression ($k1$ = 2.0237 ± 0.1025, $k2$ = –1.9958 ± 0.6033). Error estimation of the resulting software sensor was performed by 3-fold cross-validation over the data from all three cultivations. Firstly, the dataset was randomly partitioned into 3 equal sized subsamples. Of the 3 subsamples, a single subsample was retained as the validation data for testing, and the remaining 2 subsamples were used as training data. The cross-validation process was then repeated 3 times, with each of the 3 subsamples used exactly once as the validation data. For each round, a root mean square error of cross-validation (RMSECV) was computed. The 3 results were then averaged to produce a single estimation. The obtained estimation of the sensor error was below 10 % of the biomass concentration range, which is comparable to the results reported in the literature for similar types

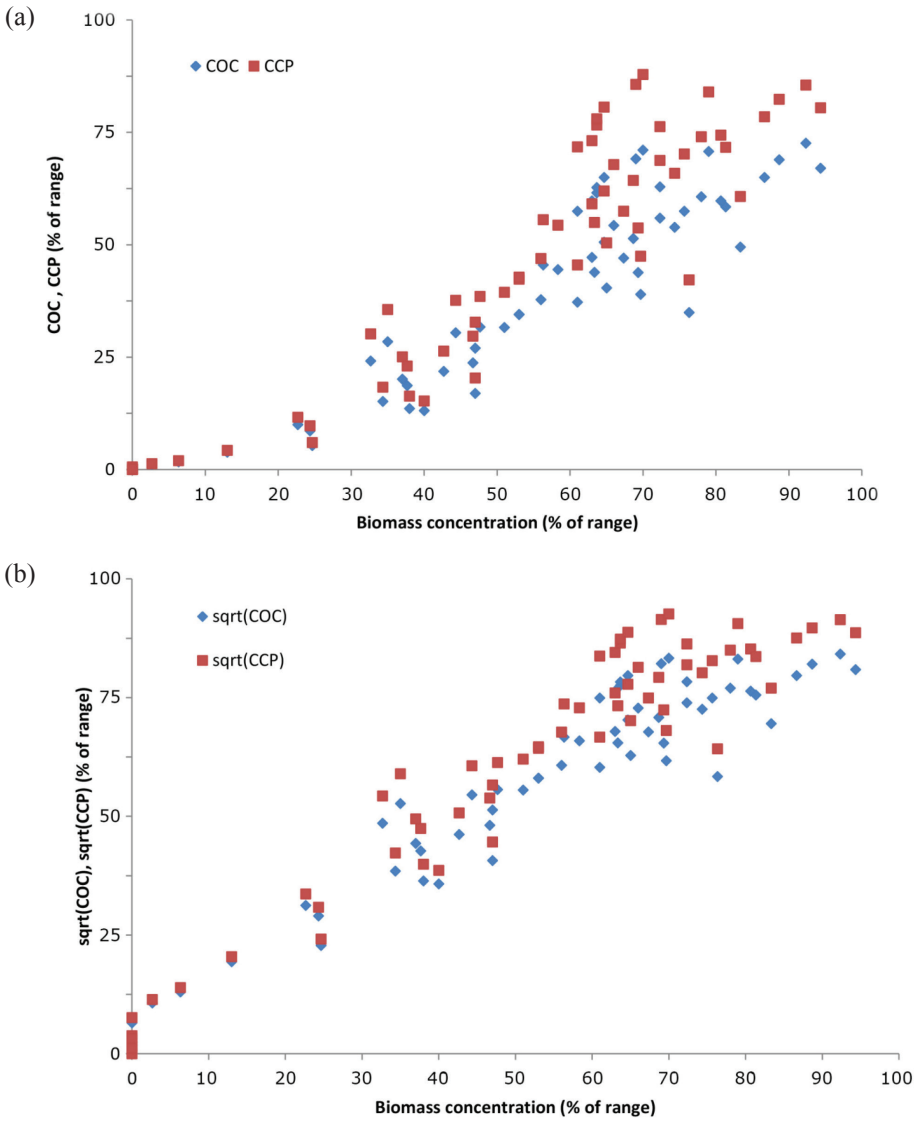


Fig. 3 – *Relationship between biomass concentration (dry cell mass) and COC, CCP (a) and sqrt(COC), sqrt(CCP) (b), respectively, data from cultivations 1 – 3*

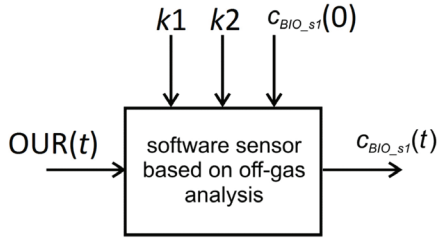


Fig. 4 – *Input-output scheme of the software sensor based on off-gas analysis*

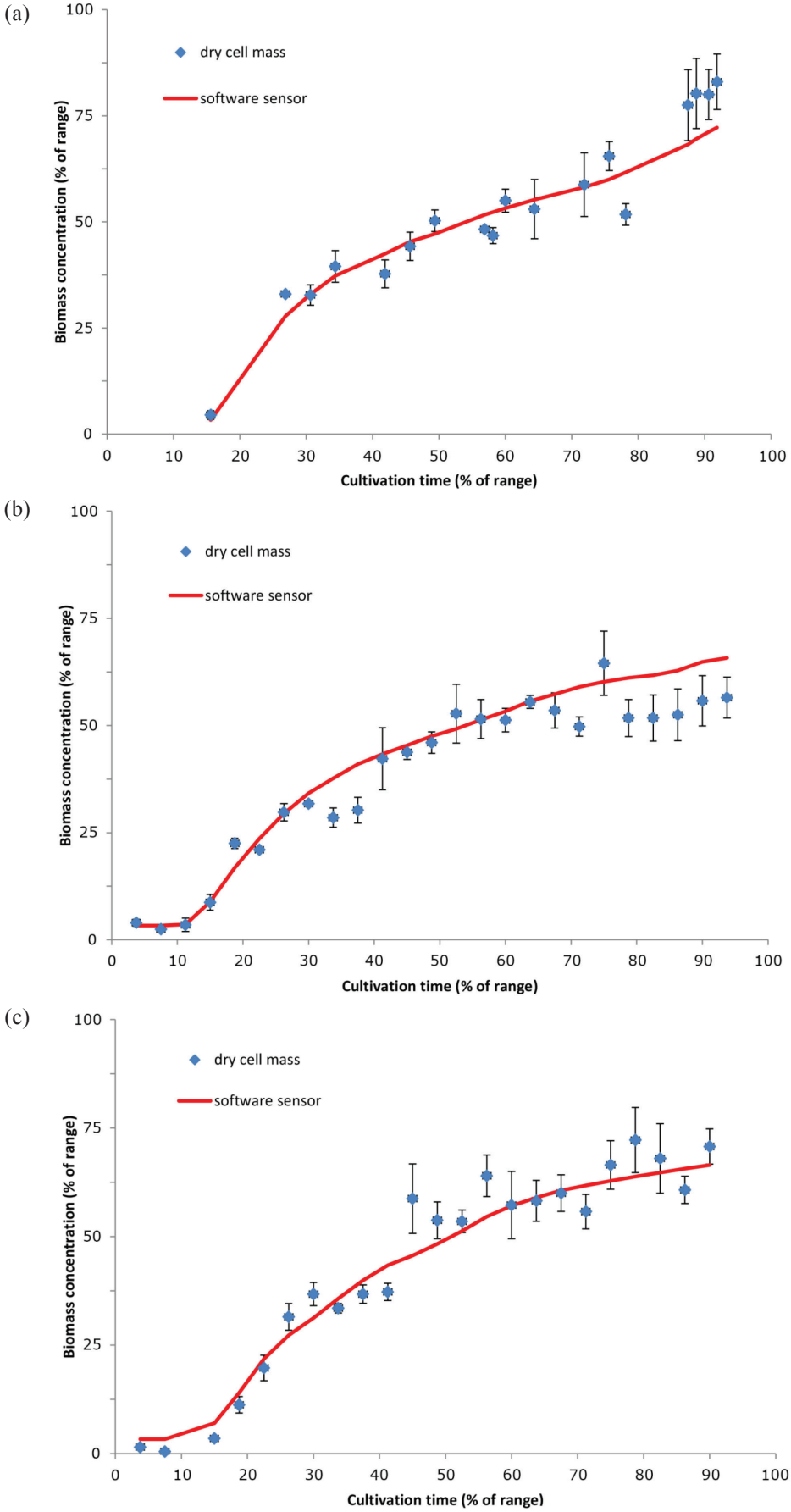
of software sensors for filamentous fermentations[16]. Fig. 5 shows the biomass concentration estimates of the software sensor for all three cultivations. As an initial estimate of the dry biomass concentration in the bioreactor at the beginning of the cultivation after inoculation, the value of 1.33 ± 0.36 kg m^{-3} (average $c_{BIO\_S1}(0)$ over all three cultivations) was used.

Biomass concentration estimation using software sensor based on calorimetry

Specific bioprocess heat production

Using Eq. 8, more than 20 experimental data series obtained during the cultivation C1 using the laboratory calorimeter were evaluated, and the Q_{CAL} values shown in Fig. 6 were obtained.

However, since the biomass culture conditions were not maintained at optimum level throughout the duration of the cultivation (see Results and discussion, subsection Combining off-gas analysis and calorimetry data for process state monitoring), the biomass did not produce as much bioprocess heat as it would under optimal conditions. For this reason, the upper envelope curve of the data, shown in Fig. 7, was taken into account. Linear regression using least square minimization was then used to fit the



F i g . 5 – *Estimation of biomass concentration by software sensor from on-line measured off-gas composition data in cultivations C1–3 (a, b, c) compared to the dry biomass mass values determined by gravimetric analysis*

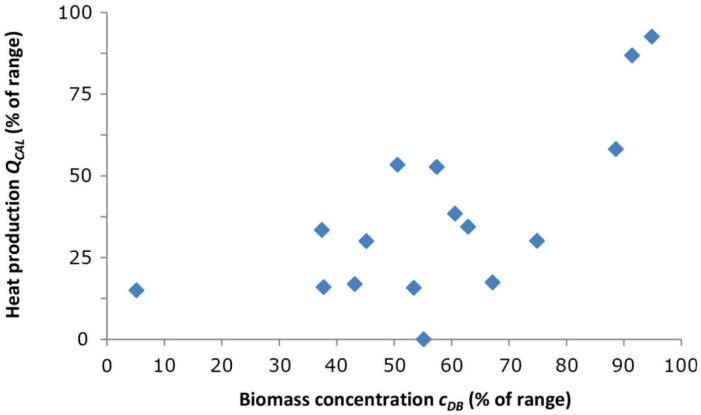


Fig. 6 – *Results of calorimetric measurements in the laboratory calorimeter taken during the cultivation C1*

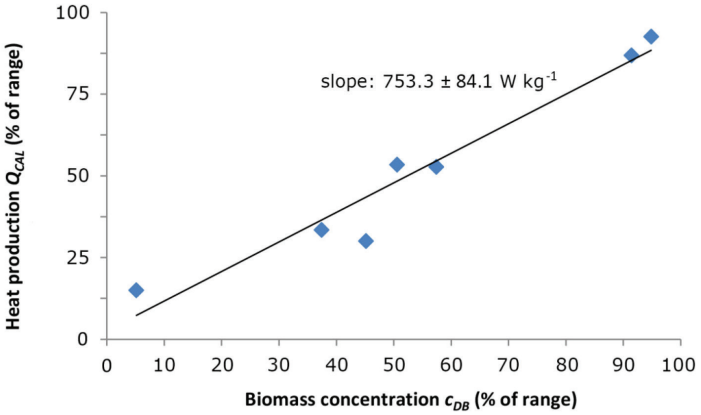


Fig. 7 – *Upper envelope of the data on the biomass heat production resulting in the average value of the specific biomass heat production equal to 753.3 ± 84.1 W kg^{-1} of dry biomass*

selected points. Standard deviation, calculated from the value differences of the points in Fig. 7 from the regression line, was used to determine the range of uncertainty (±84.1 W kg^{-1}).

Estimation of biomass concentration and the accuracy requirements on the measured process parameters

Evaluating Q_{BIO} according to Materials and methods (subsection Software sensors based on calorimetry), we can estimate the biomass concentration as follows:

$$c_{BIO\_s2}(t) = \frac{1}{V_L} \cdot \frac{Q_{BIO}(t)}{q_{sbio}} \tag{11}$$

where the value q_{sbio} is known from the measurement in laboratory calorimeter (see Materials and methods, subsection Calorimetry measurements and Fig. 8). However, maintaining the optimal cultivation conditions in the bioreactor is a prerequisite for using this equation to estimate the biomass concentration.

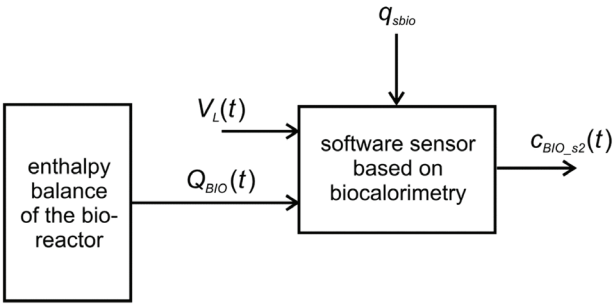


Fig. 8 – *Input-output scheme of the software sensor based on biocalorimetry*

The accuracy of the biomass concentration estimate using software sensor based on biocalorimetry additionally depends on the accuracy of the measurement of several process parameters. The following table (Table 1) shows the accuracy of individual process variables necessary to remain at a certain level of the contribution to the overall relative error of the software sensor. The necessary accuracies were obtained by the parametric study, where individual variables were changed and the effect on the resulting Q_{BIO} value was calculated in terms of the percentage of Q_{BIO} change.

Combining off-gas analysis and calorimetry data for process state monitoring

From the comparison of the time courses of the carbohydrate substrates concentration in the bioreactor and the calorimetric measurements, it is apparent that the heat production was reduced in the part of the cultivation where the substrate concentration was very low (shown in Fig. 9), at a level which is undesirable from the perspective of production. In this context, an alternative use of the proposed calorimetry software sensor is offered – the output of this sensor combined with the output of the biomass software sensor based on off-gas analysis can be used to monitor the feeding status of the production filamentous culture. In particular, the ratio of both estimates given by Eq. 12 can serve as an indicator of underfeeding (see Fig. 10), and its decrease below a suitably chosen threshold, can serve as a timely warning of the risk of onset of this undesired condition. This indicator can serve as a suitable supplement to the standard operation procedure for the monitoring of the nutritional status of the filamentous culture, which consists of laboratory off-line determination of carbohydrate substrates concentration in the bioreactor, measured as reducing substances.

$$R_{cal/oxy} = \frac{c_{BIO\_s2}}{c_{BIO\_s1}} \tag{12}$$

Table 1 – *Accuracies of individual process variables contributing to the overall relative error of the software biocalorimetry-based sensor as obtained by the parametric study*

Accuracy of each measured process variable necessary to contribute to the overall relative error of SW sensing no more than by the percentage given below								
quantity	value	unit	1 %	2 %	4 %	6 %	8 %	10 %
$T_{in,w}$	17	°C	0.039	0.078	0.16	0.23	0.31	0.39
$T_{out,w}$	24	°C	0.039	0.078	0.16	0.23	0.31	0.39
$\dot{V}_W$	6.613757	kg s^{-1}	0.094	0.19	0.38	0.57	0.76	0.94
	23.80952	m^3 h^{-1}	0.34	0.68	1.36	2.04	2.73	3.41
P_{imp}	60	kW	4	8	16	24	32	40
pw^0	3.78	kPa	0.47	0.95	1.89	2.84	3.78	4.73
T_L accuracy resulting from pw^0			2.19	4.39	8.78	13.17	17.56	21.95

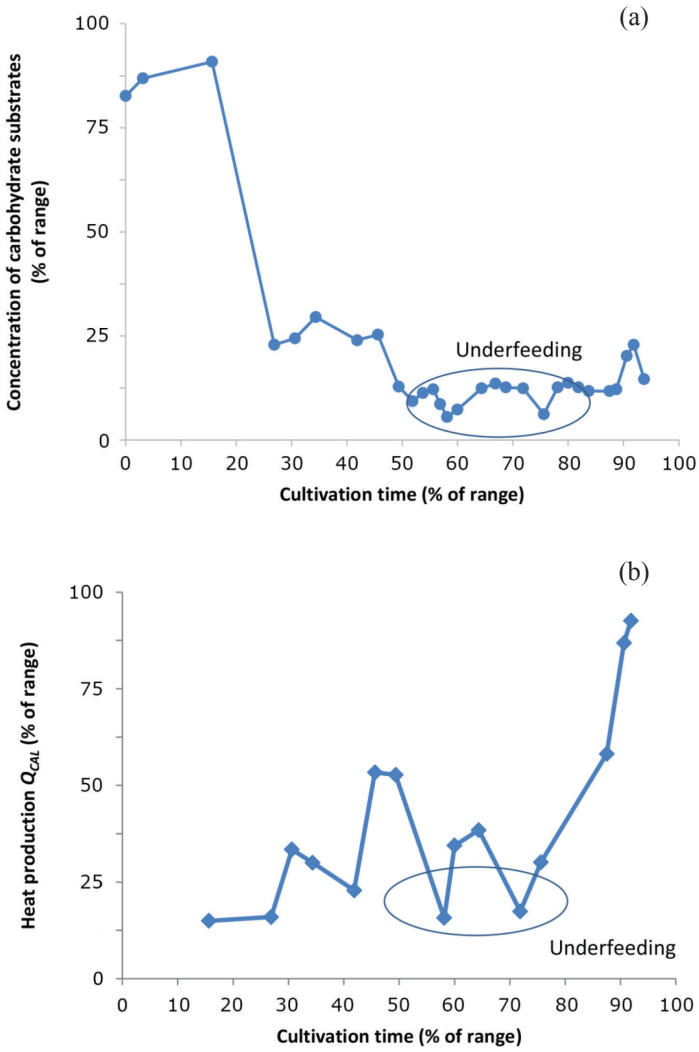


Fig. 9 – *Concentration of carbohydrate substrates in the fermentation broth measured as reducing substances (a) and calorimetric measurements (b) in cultivation C1*

Conclusions

The aim of this study was to explore the potential of two different approaches to the task of on-line estimation of biomass concentration in a filamentous microorganism cultivation process by software sensors. Whereas the first approach is based on the on-line measurement of the composition of the bioreactor off-gases (oxygen consumption and carbon dioxide production respectively), the second approach uses the on-line measurement of process variables related to the enthalpy balance of the bioreactor (calorimetry). In the case of the software sensor based on off-gas analysis, the experimental results have shown that there is a linear relationship between the biomass concentration and the square roots of cumulative consumption (oxygen) and production (carbon dioxide), respectively. Since slightly better results were obtained in the case of the cumulative oxygen consumption, the first proposed software sensor was based on this on-line measured process variable. In the case of the second explored type of software sensor – the calorimetry-based software sensor, the experimental determination of the specific bioprocess heat production was shown, and analysis performed to determine the accuracy requirements of the measured process variables. However, in the case of non-optimal conditions for biomass growth (underfeeding), the obtained experimental results have shown that the amount of heat produced per unit of biomass can be lower, and the software sensor can thus underestimate the biomass concentration. Even though this fact considerably limits the potential of the calorimetry-based software sensor for biomass concentration estimation, its alternative use can be found in the monitoring of the feeding status of the produc-

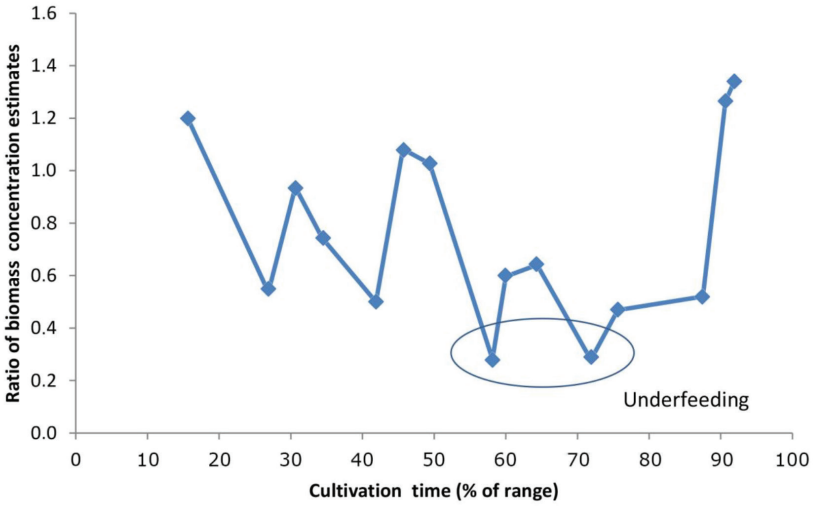


Fig. 10 – *Ratio of biomass concentration estimates based on calorimetry and off-gas composition analysis in cultivation C1*

tion filamentous culture when the outputs of both sensors are combined. Specifically, the resulting ratio can serve as an indicator of underfeeding, thus providing a useful supplement to current practice based on off-line laboratory analysis of the nutrient concentration in the bioreactor.

ACKNOWLEDGEMENTS

This study was supported by the Technology Agency of the Czech Republic under the contract No. TA03010165 "Knowledge-based control of biotechnological production of antibiotics Nystatin". This support is gratefully acknowledged.

Nomenclature

A_{surf} – surface area of the fermenter available for free convection heat exchange, m^2

CCP – cumulative carbon dioxide production, kg m^{-3}

c_{BIO_s1} – estimated biomass concentration (dry biomass) using the software sensor based on off-gas analysis, kg m^{-3}

c_{BIO_s2} – estimated biomass concentration (dry biomass) using the software sensor based on calorimetry, kg m^{-3}

c_{DB} – biomass concentration (dry biomass), kg m^{-3}

c_X – microbial cell concentration in the bioreactor, kg m^{-3}

c, c_p – heat capacity, J kg^{-1} K^{-1}

CO_2 – carbon dioxide concentration in the off-gas, % vol.

COC – cumulative oxygen consumption, kg m^{-3}

CPR – carbon dioxide production rate, kg m^{-3} s^{-1}

ΔO_2 – difference between oxygen concentrations in the inlet air and the off-gas, % vol.

ΔCO_2 – difference between carbon dioxide concentrations in the inlet air and the off-gas, % vol.

Fk – nutrient flow flag in k-th addition defined in Eq. 3

Δh – fermentation broth depth, m

ΔH_{evap} – heat of evaporation, J mol^{-1}

$k1$, $k2$ – calibration parameters of the software sensor based on off-gas analysis

k_{conv} – coefficient for the conversion of concentration values from volume percent into dimensionless volume fraction (k_{conv} = 0.01)

M – molecular weight, kg mol^{-1}

$\dot{m}$ – mass flow, kg s^{-1}

m_C – carbon dioxide coefficient related to maintenance, kg (CO_2) kg^{-1} (biomass) min^{-1}

m_{DB} – weight of dry biomass, kg

m_L – weight of fermentation broth, kg

m_O – oxygen coefficient related to maintenance, kg (O_2) kg^{-1} (biomass) min^{-1}

N_2 – nitrogen concentration in the air (assumed constant at 79.07 %), % vol.

OUR – oxygen uptake rate, kg m^{-3} s^{-1}

O_2 – oxygen concentration in the off-gas, % vol.

P_{imp} – impeller power, W

p – pressure, Pa

p_{CO_2}, p_W – partial pressure of CO_2 and water in gas, respectively, Pa

p_w^0 – water vapour pressure, Pa

Q_{CAL} – heat production in laboratory calorimeter, W

q_{bio} – specific bioprocess heat production, W kg^{-1} of fermentation broth

q_{sbio} – specific biomass heat production, W kg^{-1} of dry biomass

Q_{BIO} – bioprocess heat production in the bioreactor, W

$R_{cal/oxy}$ – ratio of outputs from software sensors based on calorimetry and off-gas analysis

t – current cultivation time, min

T – temperature, °C or K

V – volume, m^3

$\dot{V}$ – volumetric flow, $m^3\ s^{-1}$

V_{CAL} – fermentation broth sample volume in the laboratory calorimeter, L

$Y_{O/X}$ – yield coefficient relating oxygen consumption to biomass production, kg (O_2) kg^{-1} (biomass)

$Y_{C/X}$ – yield coefficient relating carbon dioxide production to biomass production, kg (CO_2) kg^{-1} (biomass)

α – heat transfer coefficient from the fermenter surface to ambient air, $W\ m^{-2}\ K^{-1}$

ρ – density, $kg\ m^{-3}$

τ – variable of integration (takes on values from time 0 to the present t), min

Subscripts

Air – physical quantities for gas phase in fermentation

ambient – physical quantities for the environment surrounding the bioreactor

in – input stream

out – output stream

surf – fermentation vessel surface

w – physical quantities for cooling water

L – physical quantities for fermentation broth

References

1. *Kadlec, P., Gabrys, B., Strandt, S.*, Data-driven soft sensors in the process industry, Comput. Chem. Eng. **33** (2009) 795. doi: https://doi.org/10.1016/j.compchemeng.2008.12.012
2. *Luttmann, R., Bracewell, D. G., Cornelissen, G., Gernaey, K. V., Glassey, J., Hass, V. C., Kaiser, C., Preusse, C., Striedner, G., Mandenius, C. F.*, Soft sensors in bioprocessing: A status report and recommendations, Biotechnol. J. **7** (2012) 1040. doi: https://doi.org/10.1002/biot.201100506
3. *Sharma, S., Tambe, S. S.*, Softsensor development for biochemical systems using genetic programming, Biochem. Eng. J. **85** (2014) 89. doi: https://doi.org/10.1016/j.bej.2014.02.007
4. *Aehle, M., Kuprijanov, A., Schaepe, S., Simutis, R., Luebbert, A.*, Simplified off-gas analyses in animal cell cultures for process monitoring and control purposes, Biotechnol. Lett. **33** (2011) 2103. doi: https://doi.org/10.1007/s10529-011-0686-5
5. *Chéruy, A.*, Software sensors in bioprocess engineering, J. Biotechnol. **52** (1997) 193. doi: https://doi.org/10.1016/S0168-1656(96)01644-6
6. *Montague, G. A., Morris, A. J., Tham, M. T.*, Enhancing bioprocess operability with generic software sensors, J. Biotechnol. **25** (1992) 183. doi: https://doi.org/10.1016/0168-1656(92)90114-O
7. *Ignova, M., Glassey, J., Ward, A. C., Montague, G. A.*, Multivariate statistical methods in bioprocess fault detection and performance forecasting, Trans. Inst. MC **19** (1997) 271. doi: https://doi.org/10.1177/014233129701900507
8. *Albiol, J., Robustr, J., Casas, C., Poch, M.*, Biomass estimation in plant cell cultures using an extended Kalman filter, Biotechnol. Prog. **9** (1993) 174. doi: https://doi.org/10.1021/bp00020a009
9. *Arnold, S. A., Crowley, J., Woods, N., Harvey, M. L.*, In-situ near infrared spectroscopy to monitor key analytes in mammalian cell cultivation, Biotechnol. Bioeng. **84** (2003) 13. doi: https://doi.org/10.1002/bit.10738
10. *Faergestad, E. M., Oyaas, J., Kohler, A., Berg, T., Naes, T.*, The use of spectroscopic measurements from full scale industrial production to achieve stable end product quality, J. Food. Sci. Technol. **44** (2011) 2266.
11. *Gao, Y., Yuan, Y. J.*, Comprehensive quality evaluation of corn steep liquor in 2-keto-L-gulonic acid fermentation, J. Agric. Food. Chem. **59** (2011) 9845. doi: https://doi.org/10.1021/jf201792u
12. *Cunha, C. C. F., Glassey, J., Montague, G. A., Albert, S., Mohan, P.*, An assessment of seed quality and its influence on productivity estimation in an industrial antibiotic fermentation, Biotechnol. Bioeng. **78** (2002) 658. doi: https://doi.org/10.1002/bit.10258
13. *Jenzsch, M., Simutis, R., Eisbrenner, G., Stückrath, I., Lübbert, A.*, Estimation of biomass concentrations in fermentation processes for recombinant protein production, Bioprocess. Biosyst. Eng. **29** (2006) 19. doi: https://doi.org/10.1007/s00449-006-0051-6
14. *Ödman, P., Lindavald Johansen, C., Olsson, L., Gernaey, K. V., Eliasson Lantz, A.*, On-line estimation of biomass, glucose and ethanol in *S. cer.* cultivations using in-situ multi-wavelength fluorescence and software sensors, J. Biotechnol. **144** (2009) 102. doi: https://doi.org/10.1016/j.jbiotec.2009.08.018
15. *Sundström, H., Enfors, S.*, Software sensors for fermentation processes, Bioprocess. Biosyst. Eng. **31** (2008) 145. doi: https://doi.org/10.1007/s00449-007-0157-5
16. *Rønnest, N. P., Stocks, S. M., Eliasson Lantz, A., Gernaey, K. V.*, Introducing process analytical technology (PAT) in filamentous cultivation process development: Comparison of advanced online sensors for biomass measurement, J. Ind. Microbiol. Biotechnol. **38** (2011) 1679.
17. *Turker, M.*, Development of biocalorimetry as a technique for process monitoring and control in technical scale fermentations, Thermochim. Acta **419** (2004) 73. doi: https://doi.org/10.1016/j.tca.2004.01.036
18. *Voisard, D., Pugeaud, P., Kumar, A. R., Jenny, K., Jayaraman, K.*, Development of a large-scale biocalorimeter to monitor and control bioprocess, Biotechnol. Bioeng. **80** (2002) 125. doi: https://doi.org/10.1002/bit.10351
19. *Randolph, T. W., Marison, I. W., Berney, C., von Stockar, U.*, Bench-scale calorimetry of hybridomass in suspension culture, Biotechnol. Tech. **3** (1989) 369. doi: https://doi.org/10.1007/BF01875001