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Mathematical Description of Functional States in *E. coli* Fed-batch Cultivation Processes

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Abstract: *This paper presents an overview of a biochemical correspondence to defined functional states based on specific metabolic mechanisms. For Escherichia coli fed-batch cultivation processes a set of four functional states is considered. It is shown that the process can be divided into several functional states by considering the cell metabolism in more detail. As a result through the proposed functional states the changes in metabolic pathways can be described accurately. For each functional state a respective local model is proposed. Simulations of the E. coli cultivation process with functional state modelling are presented. Furthermore different functional states in a real E. coli MC4110 fed-batch cultivation process are identified and local models are developed. By simulations and comparing results to experimental data is shown that the concept of functional state modelling works in practice and leads to more precise and adequate mathematical description.*

Keywords: *Functional states, Metabolism, Mathematical description, Fed-batch processes, Escherichia coli.*

Introduction

The mathematical description of a cultivation process on the basis of a number of separate functional states has been shown useful in modelling and control of such complex processes [8, 15, 23, 24]. In each functional state (top level of the system hierarchy) the process is described by a conventional type of model, called local model, which is valid only in this functional state. At the second hierarchical level some numeric detection algorithms and/or rules based on expert knowledge can be used for the recognition of the functional state and state transitions. A set of local models together with functional state "dynamics" can be used to describe, monitor and control the overall growth process.

Murray-Smith and Johansen [12] have provided an introduction to the functional state concept and have illustrated it with a simple wire model. The methods for detection of changes in process dynamics and transitions between states are applied to a practical biotechnical process control task. One of the most important characteristics of biotechnological processes, which make the control design more difficult, is the change of cell population state. In most cases this change is expected in view of the fact that the cells pass through different phases and growth states. In many batch-type cultivation processes, the functional states would naturally be identified with the different phases of the process – lag phase, log phase, stationary phase and death phase. In a fed-batch or continuous process the main problems are how to divide the

process into macrostates – appropriate functional states, and to detect when the process is in a certain functional state. Based on the physiological state control concept [7], fuzzy rules can be used to describe memberships of current process states to certain process phases. For each phase a certain feeding profile or controller set points have to be designed. The research of Ruenglertpanyakul and Bellgardt [25] introduces an approach, based on an expert system, for developing model of bioprocesses. The expert system is used to develop physiological phase models, which is valid only in one physiological phase, as well as the switching conditions from one to another phase. The expert system was tested with data from cultivation of *Klebsiella terrigena*. The functional state modelling approach is applied by Zhang et al. [28] for aerobic yeast growth process. The authors presented on-line recognition of functional states and use the results to demonstrate an optimal substrate feed control policy [28, 29].

In this paper the functional state modelling approach in connection with *Escherichia coli* fed-batch cultivation processes is applied. A division of the *E. coli* growth process into several functional states is discussed. According to the bacteria's metabolism and dominated metabolic pathways in each functional state mathematical description (local models) is proposed.

Mathematical description of functional states

Based on known analogies between metabolic mechanisms of yeast and *E. coli* [23] and according to [21] the whole bacteria growth process can be divided into four functional states in fed-batch cultures:

- *First acetate production state (FS I)*;
- *Mixed oxidative state (FS II)*;
- *Complete sugar oxidative state (FS III)*;
- *Second acetate production state (FS V).*

Each functional state is characterized with specific biochemical processes and could be mathematically described. Mathematical models are developed according to the mass balance of the systems. Considering an *E. coli* fed-batch cultivation process mathematical descriptions for different functional states are presented below.

The general state space dynamical model described by Bastin and Dochain [1] is accepted as representing the dynamics of an *n* components and *m* reactions bioprocess:

$$\frac{dx}{dt} = K\varphi(x,t) - Dx + F - Q\,, \tag{1}$$

where x is a vector representing the state components; K is the yield coefficient matrix; φ is the growth rates vector; the vectors F and Q are the feed rates and the gaseous outflow rates. The scalar D is the dilution rate, which will be the manipulated variable, defined as follows:

$$D = \frac{F_{in}}{V}\,, \tag{2}$$

where $F_{in,}$ [l/h] the influent flow rate and V, [l] the bioreactor volume.

First acetate production state (FS I)

The rules for recognition of *FS I* during the *E. coli* cultivation process are:

$$S > S_{crit}\,,\ pO_2 > pO_{2crit} \text{ and } A > 0\,, \tag{3}$$

where S is concentration of substrate (glucose), [g/l]; pO_2 – concentration of dissolved oxygen, [%]; A – concentration of acetate, [g/l]; S_{crit} and pO_{2crit} – critical values of substrate and dissolved oxygen concentrations.

The growth of *E. coli* in this functional state could be represented through the reaction:

$$k_1 S + k_8 pO_2 \xrightarrow{\mu_1} X + k_5 A, \quad (4)$$

where X is biomass concentration, [g/l]; k_1, k_5 and k_8 are reaction coefficients, [-] and μ_1 is reaction rate, [h^{-1}].

In this state the main carbon source is the glucose and the acetate excretion is the result of a metabolic "overflow" mechanism [21]. Overflow metabolism has been attributed to an enzymatic limitation in the TCA cycle. If the rate of dissolved oxygen utilization is sufficiently high, the reduced cofactors generated by glucose consumption are reoxidized in the electron transport chain, which serves the dual purpose of maintaining an optimal redox environment and generating energy by oxidative phosphorylation. If the rate of glucose consumption is greater than the capacity to re-oxidize the reduced equivalents, metabolic intermediates accumulate to maintain the redox balance [27].

Mixed oxidative state (FS II)

The process enters this state when the following conditions are available:

$$S \le S_{crit}, pO_2 \ge pO_{2crit} \text{ and } A > 0. \quad (5)$$

Here both sugar and produced acetate are co-metabolized through the oxidative pathways in the state. The bacteria *E. coli* are able to re-oxidize the reduced metabolic intermediates that were accumulated during the first functional state. In this functional state accumulated acetate is also metabolized. Therefore the growth process could be described through the next two reactions:

$$\begin{aligned} k_2 S + k_9 pO_2 &\xrightarrow{\mu_{2S}} X \\ k_6 A + k_{10} pO_2 &\xrightarrow{\mu_{2A}} X, \end{aligned} \quad (6)$$

where k_2, k_6, k_9 and k_{10} are reaction coefficients, [-] and μ_{2S}, μ_{2A} are reaction rates, [h^{-1}].

Complete sugar oxidative state (FS III)

The rules for recognition of *FS III* have to meet the requirements:

$$S \le S_{crit}, pO_2 \ge pO_{2crit} \text{ and } A = 0. \quad (7)$$

In this state, sugar is completely oxidized to water and carbon dioxide. There is sufficient quantity of dissolved oxygen in the media which helps with the reoxidizing of the metabolic intermediates in the cell (NADH and FADH). This means that there will be no acetate formation and the pyruvate will enter directly the TCA cycle and will be completely oxidized to water, carbon dioxide and several molecules of NADH and FADH. In this case the following reaction is used:

$$k_3 S + k_{11} pO_2 \xrightarrow{\mu_3} X, \quad (8)$$

where k_3 and k_{11} are reaction coefficients, [-] and μ_3 is rate of the reaction, [h^{-1}].

Second acetate production state (FS V)
The conditions for this state are:

$$S \le S_{crit}\,,\; pO_2 < pO_{2crit} \text{ and } A > 0\,. \tag{9}$$

When the dissolved oxygen becomes the limiting factor for *E. coli* growth, acetate is produced. Due to the low level of dissolved oxygen metabolic intermediates can not be reoxidized. This means that the process of glucose metabolism will stop at the stage of acetyl-coA formation by the pyruvate dehydrogenase complex. And from there the acetyl-coA will be oxidized to acetate which will be stored or excreted from the cell. This acetate will then be used for consumption by the cell if the dissolved oxygen concentration exceeds the critical minimum. The growth of *E. coli* in *FS V* could be interpreted through the following reaction:

$$k_4 S \xrightarrow{\mu_5} X + k_7 A\,, \tag{10}$$

where k_4 and k_7 are reaction coefficients, [-] and μ_5 is reaction rate, [h^{-1}].

General state space dynamical local models

The mathematical model of *E. coli* fed-batch cultivation processes can be presented by the following four submodels (local models) according to which functional state the process is:

Local model for first acetate production state (FS I):

$$\left|\begin{array}{l}
\dfrac{dX}{dt} = \mu_{max_1}\dfrac{S}{k_{S_1}+S}X - \dfrac{F_{in}}{V}X \\
\dfrac{dS}{dt} = -\dfrac{1}{Y_{S/X_1}}\mu_{max_1}\dfrac{S}{k_{S_1}+S}X + \left(S_{in}-S\right)\dfrac{F_{in}}{V} \\
\dfrac{dA}{dt} = \dfrac{1}{Y_{A/X_1}}\mu_{max_1}\dfrac{S}{k_{S_1}+S}X - \dfrac{F_{in}}{V}A \\
\dfrac{dpO_2}{dt} = -\dfrac{1}{Y_{pO_2/X_1}}\mu_{max_1}\dfrac{S}{k_{S_1}+S}X - \dfrac{F_{in}}{V}pO_2 + k_L a_1\left(pO_2^* - pO_2\right) \\
\dfrac{dV}{dt} = F_{in},
\end{array}\right. \tag{11}$$

where $\mu_1 = \mu_{max_1}\dfrac{S}{k_{S_1}+S}$, $k_1 = \dfrac{1}{Y_{S/X_1}}$, $k_5 = \dfrac{1}{Y_{A/X_1}}$, $k_8 = \dfrac{1}{Y_{pO_2/X_1}}$ and $OTR_1 = k_L a_1\left(pO_2^* - pO_2\right)$.

In this functional state the specific growth rate is described using Monod kinetics. The specific rate of sugar consumption, the specific acetate rate and the specific dissolved oxygen consumption rate are directly proportional to the specific growth rate, i.e. to the Monod kinetics.

Local model for mixed oxidative state (FS II):

$$\begin{aligned}
&\frac{dX}{dt} = \mu_{max_2}\frac{S}{k_{S_2}+S}X + \mu_{max\,A}\frac{A}{k_A+A}X - \frac{F_{in}}{V}X \\
&\frac{dS}{dt} = -\frac{1}{Y_{S/X_2}}\mu_{max_2}\frac{S}{k_{S_2}+S}X + (S_{in}-S)\frac{F_{in}}{V} \\
&\frac{dA}{dt} = -\frac{1}{Y_{A/X_2}}\mu_{max\,A}\frac{A}{k_A+A}X - \frac{F_{in}}{V}A \\
&\frac{dpO_2}{dt} = -(\frac{1}{Y_{pO_2/S_2}}\mu_{max_2}\frac{S}{k_{S_2}+S} + \frac{1}{Y_{pO_2/A_2}}\mu_{max\,A}\frac{A}{k_A+A})X - \frac{F_{in}}{V}pO_2 + \\
&\qquad + k_L a_2\left(pO_2^* - pO_2\right) \\
&\frac{dV}{dt} = F_{in},
\end{aligned} \tag{12}$$

where $\mu_{2S} = \mu_{max_2}\frac{S}{k_{S_2}+S}$, $\mu_{2A} = \mu_{max\,A}\frac{A}{k_A+A}$, $k_2 = \frac{1}{Y_{S/X_2}}$, $k_6 = \frac{1}{Y_{A/X_2}}$, $k_9 = \frac{1}{Y_{pO_2/S_2}}$, $k_{10} = \frac{1}{Y_{pO_2/A_2}}$ and $OTR_2 = k_L a_2\left(pO_2^* - pO_2\right)$.

In *mixed oxidative state* the specific growth rate is expressed according to the sum of two terms. The first term is describing the contribution of glucose and the second – the contribution of acetate to bacterial growth. Both terms have the structure of Monod kinetics. A Monod model is also used for the specific acetate and glucose rates, respectively, according to acetate concentrations and substrate concentrations. The specific dissolved oxygen consumption rate is obtained as a sum of two terms, which are directly proportional to the specific glucose rate and specific acetate rate, respectively.

Local model for complete sugar oxidative state (FS III):

$$\begin{aligned}
&\frac{dX}{dt} = \mu_{max_3}\frac{S}{k_{S_3}+S}X - \frac{F_{in}}{V}X \\
&\frac{dS}{dt} = -\frac{1}{Y_{S/X_3}}\mu_{max_3}\frac{S}{k_{S_3}+S}X + (S_{in}-S)\frac{F_{in}}{V} \\
&\frac{dA}{dt} = 0X - \frac{F_{in}}{V}A \\
&\frac{dpO_2}{dt} = -\frac{1}{Y_{pO_2/X_3}}\mu_{max_3}\frac{S}{k_{S_3}+S}X - \frac{F_{in}}{V}pO_2 + k_L a_3\left(pO_2^* - pO_2\right) \\
&\frac{dV}{dt} = F_{in},
\end{aligned} \tag{13}$$

where $\mu_3 = \mu_{max_3}\frac{S}{k_{S_3}+S}$, $k_3 = \frac{1}{Y_{S/X_3}}$, $k_{11} = \frac{1}{Y_{pO_2/X_3}}$ and $OTR_3 = k_L a_3\left(pO_2^* - pO_2\right)$.

In the *complete sugar oxidative state* the specific rates of bacterial growth, sugar consumption and dissolved oxygen consumption are described by Monod models. Here the specific acetate production rate is zero.

Local model for second acetate production state (FS V):

$$\left|\begin{array}{l} \dfrac{dX}{dt} = \mu_{max_5}\dfrac{S}{k_{S_5}+S}X - \dfrac{F_{in}}{V}X \\ \dfrac{dS}{dt} = -\dfrac{1}{Y_{S/X_5}}\mu_{max_5}\dfrac{S}{k_{S_5}+S}X + \left(S_{in}-S\right)\dfrac{F_{in}}{V} \\ \dfrac{dA}{dt} = \dfrac{1}{Y_{A/X_5}}\mu_{max_5}\dfrac{S}{k_{S_5}+S}X - \dfrac{F_{in}}{V}A \\ \dfrac{dpO_2}{dt} = 0X - \dfrac{F_{in}}{V}pO_2 + k_L a_5\left(pO_2^* - pO_2\right) \\ \dfrac{dV}{dt} = F_{in}, \end{array}\right. \tag{14}$$

where $\mu_5 = \mu_{max_5}\dfrac{S}{k_{S_5}+S}$, $k_4 = \dfrac{1}{Y_{S/X_5}}$, $k_7 = \dfrac{1}{Y_{A/X_5}}$ and $OTR_5 = k_L a_5\left(pO_2^* - pO_2\right)$

In the *second acetate production state* the specific rates of bacterial growth and acetate production depend on the glucose concentration. The specific rate of sugar consumption is described again by Monod model. Here the specific dissolved oxygen consumption rate is zero.

Simulation of the *E. coli* fed-batch cultivation process with functional state modelling

Proposed local models are used for numerical simulations of the functional states in the *E. coli* fed-batch cultivation process. For process simulation the ode45 integration algorithm is used under *Matlab 5.3* environment [26].

Functional state transitions are determined according to the conditions on the concentrations of substrate, dissolved oxygen and acetate. For considered fed-batch cultivation process it is necessary to maintain the residual substrate concentration at a very low level. A low residual level of substrate is advantageous in: (i) removing repressive effects of rapidly utilized carbon sources and maintaining conditions in the culture within the aeration capacity of the bioreactor; (ii) avoiding the toxic effects of a medium component. According to the above mentioned and based on the specific peculiarity of the *E. coli* cultivation process the value for critical substrate concentration S_{crit} = 0.02 g/l is assumed. The critical dissolved oxygen concentration is pO_{2crit} = 21%.

When the functional state is changed the local models are changed correspondingly. The initial values for simulations in the new functional state are the last simulated values in the previous functional state so that the trajectories became continuous. The parameter values used in the simulation are presented in Table 1. The parameter values are chosen based on the typical values published in the literature regarding *E. coli* fed-batch cultivation process [2, 4, 9, 10, 14].

Table 1. Parameter values used in the simulation

FS I		***FS II***		***FS III***		***FS V***	
Parameter	**Value**	**Parameter**	**Value**	**Parameter**	**Value**	**Parameter**	**Value**
μ_{max_1}, [h^{-1}]	0.32	μ_{max_2}, [h^{-1}]	0.30	μ_{max_3}, [h^{-1}]	0.50	μ_{max_5}, [h^{-1}]	0.35
k_{S_1}, [g/l]	0.02	$\mu_{max\,A}$, [h^{-1}]	0.15	k_{S_3}, [g/l]	0.03	k_{S_5}, [g/l]	0.03
Y_{S/X_1}, [-]	0.46	k_{S_2}, [g/l]	0.03	Y_{S/X_3}, [-]	0.50	Y_{S/X_5}, [-]	0.50
Y_{A/X_1}, [-]	0.10	k_A, [g/l]	0.04	Y_{pO_2/X_3}, [-]	0.50	Y_{A/X_5}, [-]	0.10
Y_{pO_2/X_1}, [-]	0.50	Y_{S/X_2}, [-]	0.49	k_La_3, [h^{-1}]	80.0	k_La_5, [h^{-1}]	30.0
k_La_1, [h^{-1}]	52.0	Y_{A/X_2}, [-]	0.09				
		Y_{pO_2/S_2}, [-]	0.20				
		Y_{pO_2/A_2}, [-]	0.40				
		k_La_2, [h^{-1}]	87.0				

Based on series of simulations a substrate feed rate profile (Fig. 1) is designed to ensure the presence of all considered functional states in the simulation. In practice it is possible a certain functional state to appear more than once and some of them do not appear at all. In this case the aim is to present the dynamics of a cultivation process during all defined functional states. The process simulation is presented in Fig. 2.

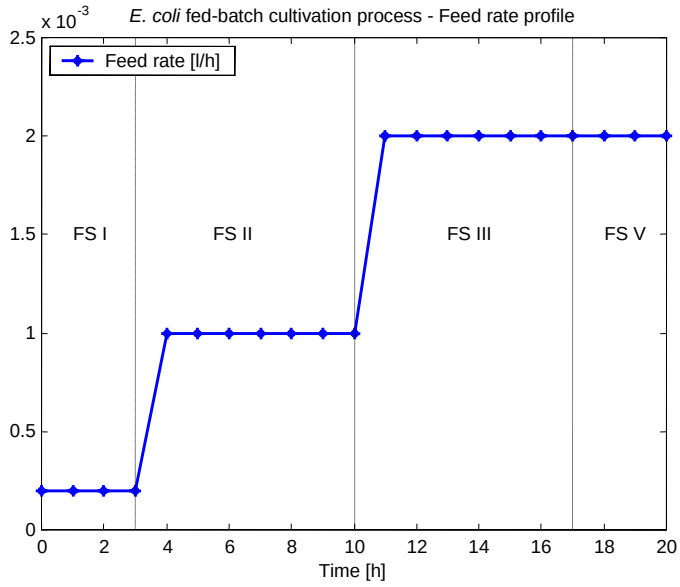

Fig. 1 Feed rate profile of *E. coli* fed-batch cultivation process

As it is presented in Fig. 2 the defined functional state (*FS I*, *FS II*, *FS III* and *FS V*) can be clearly recognized. In the first acetate production state the sugar concentration decreases very rapidly and acetate is accumulated in large quantities. The production of acetate continues until the end of the first acetate production state. In the *mixed oxidative state* bacteria begin to utilize acetate together with sugar. At the end of this state the acetate concentration is almost zero and the sugar concentration is below the critical sugar concentration. In the next state (*complete sugar oxidative state*) there is no acetate production, the bacteria uses only sugar as main energy source. In all above-mentioned functional states there is sufficient dissolved

oxygen. In the last functional state – *second acetate production state*, for second time acetate is accumulated in the system because of the insufficient level of dissolved oxygen.

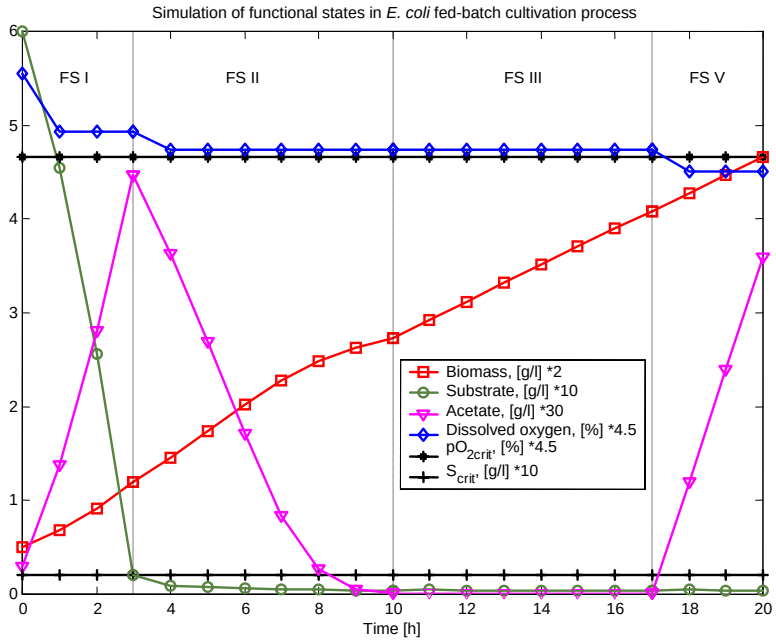

Fig. 2 Simulated *E. coli* fed-batch cultivation process using local models

Mathematical models of functional states in a real *E. coli MC4110* fed-batch cultivation process are identified too. The cultivation condition and the experimental data have been published previously [5, 22]. For the considered *E. coli* cultivation process is carried out assuming the following critical concentrations: S_{crit} = 0.1 g/l and pO_{2crit} = 21%. During the process two functional states are identified, namely *first acetate production state* (*FS I*) and *second acetate production state* (*FS V*).

Parameter estimation of the proposed local models for *FS I* and *FS V* (systems (12) and (15)) are done using genetic algorithms. Their advantages as a powerful tool for identification of strong non-linear and time-varying processes, such as cultivation processes, are well-known [3, 6, 11, 13, 16, 17]. Based on results in [17-20], genetic algorithm adjustments for considered here parameter identification are summarized in Tables 2-3.

Table 2. Genetic algorithm operators

Operator	Type
encoding	binary
crossover	double point
mutation	bit inversion
selection	roulette wheel selection
fitness function	linear ranking

Table 3. Genetic algorithm parameters

Parameter	Value
generation gap	0.97
crossover rate	0.70
mutation rate	0.05
precision of binary representation	20
number of individuals	100
number of generations	200

The optimization criterion is presented as a minimization of a distance measure J between experimental and model predicted values of state variables, represented by the vector y:

$$J = \sum_{i=1}^{n} \sum_{j=1}^{m} \left\{ \left[y_{exp}(i) - y_{mod}(i) \right]_j \right\}^2 \rightarrow min, \quad (16)$$

where y_{exp} is experimental data vector, y_{mod} – model predicted data vector, n – number of measurements for each state variable and m – number of state variables.

The estimated parameter values are listed in Table 4. Both the real cultivation trajectories and the simulated ones are shown in Fig. 3. The feed rate and initial values used in simulations are the same as in the real experiments. Again the initial values for simulations in the new state are the last simulated values in the previous one so that the trajectories became continuous.

Table 4. Estimated local models parameter values of the real *E. coli MC4110* cultivation process

FS I		***FS V***	
Parameter	**Value**	**Parameter**	**Value**
μ_{max_1}, [h^{-1}]	0.450	μ_{max_5}, [h^{-1}]	0.590
k_{S_1}, [g/l]	0.030	k_{S_5}, [g/l]	0.039
Y_{S/X_1}, [-]	0.460	Y_{S/X_5}, [-]	0.490
Y_{A/X_1}, [-]	0.019	Y_{A/X_5}, [-]	0.013
Y_{pO_2/X_1}, [-]	0.096	$k_L a_5$, [h^{-1}]	69.93
$k_L a_1$, [h^{-1}]	52.49		

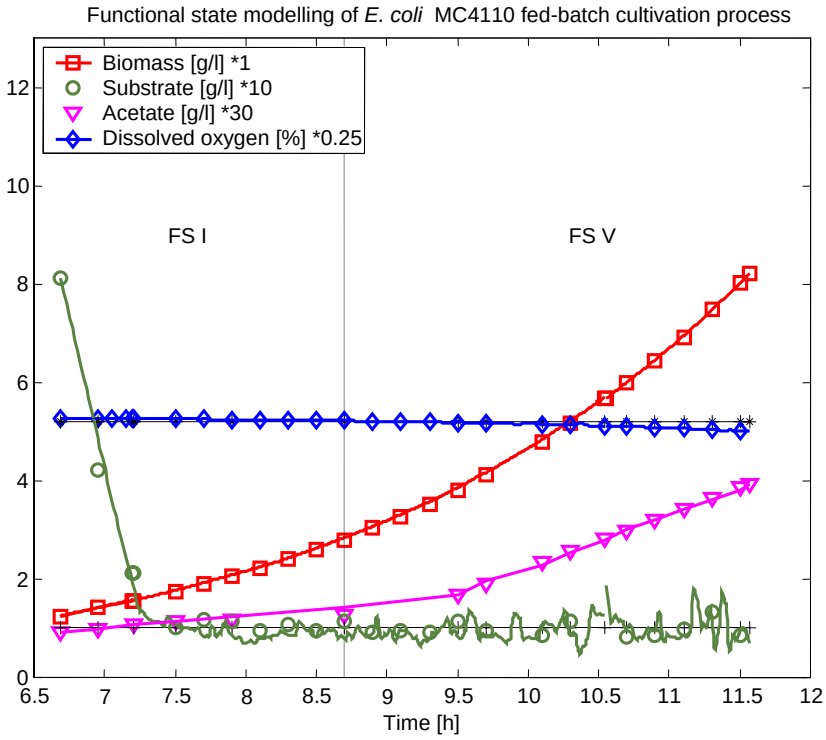

Fig. 3 Real *E. coli MC4110* cultivation trajectories (with symbols) and the simulated ones (with solid line) using local models

In this fed-batch cultivation process only two functional states appear since there is sufficient dissolved oxygen during the first four hours. In the beginning of the cultivation the dissolved oxygen and the glucose concentrations are above the corresponding critical levels. The process is in the *first acetate production state* (*FS I*). At the 8.7h of the cultivation process the concentration of dissolved oxygen becomes below the dissolved oxygen critical level and the process enters in the *second acetate production state* (*FS V*). Here the concentration of the sugar concentration is below the critical sugar concentration and the acetate production continues.

The simulation results show that the developed local models based on functional states predict successfully the variation of glucose consumption, biomass concentration, acetate formation and dissolved oxygen consumption during the fed-batch fermentation of *E. coli MC4110*. The results illustrate that the concept of functional states leads to the precise and adequate mathematical description.

Conclusion

In this paper based on an overview of specific metabolic mechanisms in *E. coli* fed-batch cultivation processes a set of four functional states is considered. By simulations it is shown that the process can be divided into several functional states by considering the cell metabolism in more detail. For each functional state a corresponding local model is developed. The functional state modelling is applied to a real *E. coli MC4110* fed-batch cultivation process. As a result different functional states are identified and local models are proposed. The results and the simulations indicate that the process can be rather well modeled with the functional state approach, show a good efficiency and the ability of the applied approach to use in practice.

The functional state concept could be applied in modelling of *E. coli* cultivation processes as an alternative for the conventional "global" modelling. It is difficult to develop "global" models, which are valid for all different process phases. Although structured models are known to describe the *E. coli* cultivation process well, they are quite complex to use in industrial-scale production in contrast to "local" models.

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