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Modelling of Fermentation Processes Based on State Decomposition

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Abstract: *This paper presents an overview of implementation of state decomposition approach to modelling of Saccharomyces cerevisiae and Escherichia coli cultivation processes. This approach, so-called functional state approach, is an alternative concept which helps in modelling and control of such complex processes like fermentation processes. The concept implementation leads to a process description with simpler and more transparent local models. The functional state approach is originally developed for yeast growth processes. Based on the similarities of main metabolic pathways of yeast and bacteria, the concept of state decomposition could be applied successfully for modelling of Escherichia coli cultivations.*

Keywords: *Modelling, Decomposition, Functional states, Local models, Saccharomyces cerevisiae, Escherichia coli.*

Introduction

Different methodologies have been employed to model or describe nonlinear behavior [29, 30]. An approach, that has been extensively used, is the development of an overall nonlinear model that performs satisfactorily through the entire operating range, i.e. global modelling. Due to the disadvantages of such approach, namely complex structure and big number of parameters, the different methods have to be searched to overcome such drawbacks. There is a strong intuitive appeal in building systems which operate robustly over a wide range of operating conditions by decomposing them into a number of simpler modelling or control problems. This appeal has been a factor in the development of increasingly popular multiple-model approach, and in particular – modelling approach based on functional state decomposition, to cope with strongly nonlinear and time-varying systems [17, 34].

State decomposition of the problem into sub-problems, which can be solved independently, is one standard approach when complicated problems have to be solved. The decomposition of the system full range of operation into a number of possibly overlapping operating regimes is illustrated in Fig. 1. If fermentation processes are being considered, the partition based on glucose and dissolved oxygen concentration splits the set of operating regimes as presented in Fig. 2.

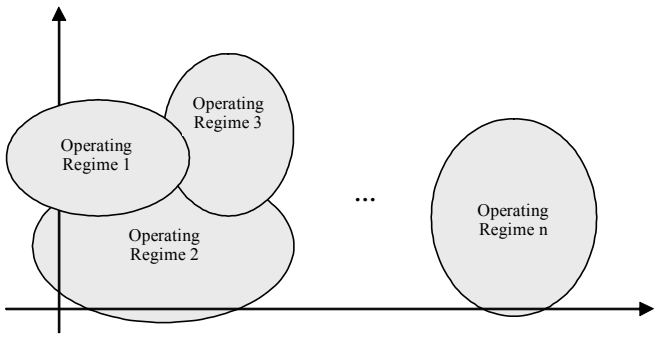

Fig. 1

In each operating regime, a simple local model could be applied. These local models are then combined in some way to yield a global model. In this way the individual solutions of the problem lead to the global solution of the complex problem. Hence, model development within this framework typically consists of the following tasks:

- Decompose the system full range of operation into operating regimes.
- Select simple local model structures within each operating regime. These structures will be often determined by the relevant system knowledge that is available under different operating conditions, as well as the intended purpose of the model.
- The local model structures are usually parameterized by certain variables that must be determined.
- A method for combining the local models into a global one must be applied. Numerous approaches exist and can be characterized according to deterministic vs. stochastic assumptions, soft or hard partitions etc.

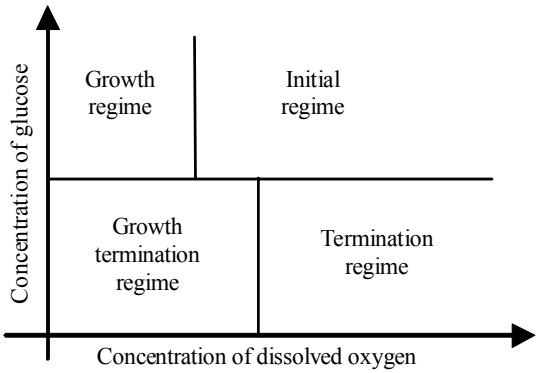

Fig. 2

In natural problems it will not be always easy to find a natural sequence in which these tasks should be approached. Several iterations of the same tasks are usually needed before a satisfactory model to be found. The main challenges in the development of dynamic models are:

- *Division of the operating space into operating regimes;*
- *Construction of local dynamic models;*
- *Aggregation of local models through a suitable switching strategy.*

These challenges have been subject of many investigations in the field of fermentation processes technology. Murray-Smith and Johansen [17] have provided an introduction to the

functional state concept and have illustrated it with a simple wire model. Implementation of advanced control strategies in bioprocesses is often hindered by the lack of on-line measurements reflecting the physiological state of the culture. Although a number of techniques have been used to estimate key variables from on-line data monitored, these often do not explicitly take into account changes in physiological state and information on many aspects of physiological state may not be present in on-line data [8]. Feng and Glassey have demonstrated [8] that data obtained from chemical fingerprinting methods, such as pyrolysis mass spectrometry; can be used to identify changes in the physiological state during cultivation. This information can be utilized for the estimation of the physiological state and can enable physiological-state-specific model development for on-line bioprocess control. Knop et al. [16] have stated that the goal of physiological-state control systems is to maintain key metabolic variables on an optimal trajectory throughout the fermentation. This investigation describes the use of a physiological state control algorithm for a high-cell-density *E. coli* fermentation to produce quinic acid from glucose. There are a few reports on extending metabolic flux analysis to on-line state recognition of fermentation processes, using only measured values acquired on-line. In research study of Takiguchi et al. [27] metabolic flux analysis is extended to an on-line approach. The fluxes of not only extracellular but also intracellular metabolites are calculated in lysine fermentation by *Corynebacterium glutamicum* for physiological state recognition. During the course of the study Fukudome et al. [10] have found that the alcian blue adsorption to yeast cell, defined as the alcian blue retention ratio, varied according to the culture conditions. These results suggest that the alcian blue retention ratio will be useful for the evaluation of yeast physiological states. In the research of Tartakovsky et al. [28] two novel approaches for modelling processes are suggested and applied to *E. coli* fermentations. The first approach uses a multi-compartment model framework, coupled with knowledge-based logic. In the second approach the multi-compartment model is reduced into the variable structure model consisting of a battery of alternative submodels, each of which qualitatively represents one of the process steps. 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 is tested with data from cultivation of *Klebsiella terrigena*. Shimizu et al. [26] have studied on-line state recognition in a yeast fed-batch culture with neural networks. Aguilar-Martin et al. [1] have described the application of a self learning algorithm generating the classes that correspond to physiological states in a bioreactor. The methodology is applied to *Saccharomyces cerevisiae* production process in oxidative regime without ethanol production. The proposed algorithm appeared to be particularly helpful for a biotechnological process on behalf to the possibility of having a good dialog with the experts, as well as sufficient off-line data for the learning period. Intuitively appealing nature of the framework is demonstrated by Murray-Smith and Johansen [17] with applications of local methods to problems in the process industries, biomedical applications and autonomous systems.

Taking into account all application of modelling approach, based on state decomposition, for fermentation processes and reported results it is obviously that the implementation of such approach has computational advantages and allows direct incorporation of high-level and qualitative plant knowledge into the model. These advantages have proven to be very appealing for industrial applications. Due to these opportunities, the aim of this paper is to present a theoretical basement and prerequisites for the application of state decomposition approach for modelling of *Escherichia coli* cultivation, based on functional state modelling of *Saccharomyces cerevisiae*.

Application of functional state modelling approach to *Saccharomyces cerevisiae* cultivation

Successful applying of functional state modelling approach requires the specific peculiarities and mechanisms of the yeast growth processes to be preliminary clarified. *Saccharomyces cerevisiae* cultivation, as typical representative of yeast, is here considered. *S. cerevisiae* uses three major pathways for growth on glucose, namely *fermentation of glucose, oxidation of glucose* and *oxidation of ethanol* [6].

- *Fermentation of glucose* occurs when glucose concentration is high and/or oxygen supply is limited:
  $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{energy}$
- *Oxidation of glucose* occurs when glucose concentration is low and oxygen supply is sufficient:
  $C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2OH + 6CO_2 + \text{energy}$
- *Oxidation of ethanol* occurs when glucose concentration is high and/or oxygen supply is limited:
  $C_2H_5OH \rightarrow 2H_2O + 2CO_2 + \text{energy}$

A substrate such as sugar is degrading by yeast to produce a number of carbon intermediates as well as to provide some reducing power and energy. Yeast then utilizes the carbon intermediates in order to synthesize new cell material. If the sugar concentration during an aerobic yeast growth process exceeds some critical level, a part of the sugar is metabolized to ethanol. This is because much pyruvate is accumulated in the cell during sugar glycolysis that cannot be completely oxidized in the tricarboxylic acid cycle. The surplus pyruvate is reduced to ethanol through the reoxidation of NADH that is produced in the latter stages of glycolysis. The yeast can also produce ethanol under aerobic conditions if there is high specific rate of growth or low dissolved oxygen concentration. In order to be more evident, a metabolic flux model of *S. cerevisiae* is presented in Fig. 3.

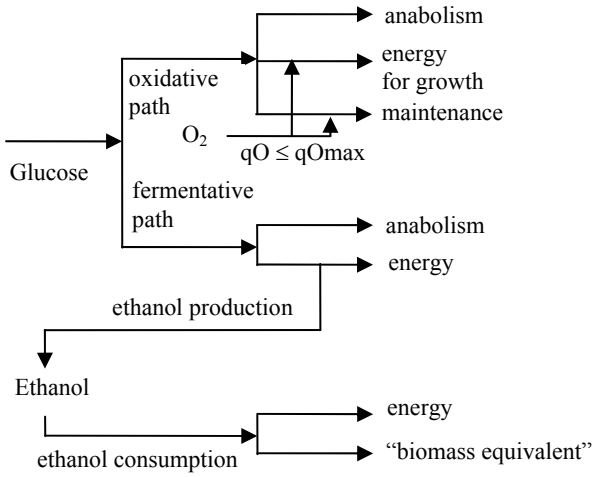

Fig. 3

As it is obvious, there are metabolic changes during the process that determine application of functional state modelling approach. The main idea of such approach is the process to be divided into macro-states, called *functional states* (*FS*), according to behavioral equivalence. In each *FS* certain metabolic pathways are active enough to dominate the overall behavior of the process. Based on the presented metabolic mechanisms and a lot of investigations, Zhang

et al. [34] have supposed that the whole yeast growth process can be divided into at least five functional states in batch and fed-batch cultures. In each functional state the yeast metabolism is dominated by certain metabolic pathways.

First ethanol production state (FS I)
The process is defined to be in this state when the sugar concentration is above the critical level and there is sufficient dissolved oxygen. In this state ethanol is produced.
Mixed oxidative state (FS II)
The process enters this state when the sugar concentration decreases to be equal to or below the critical level and there is sufficient dissolved oxygen in the broth. The process remains in this state as long as these conditions are met. Both sugar and produced ethanol are co-metabolized through the oxidative pathways in the state.
Complete sugar oxidative state (FS III)
The process is defined to be in this state when there is no ethanol available, the sugar concentration is not higher than the critical level and the dissolved oxygen is above its critical level. In this state, sugar is completely oxidized to water and carbon dioxide.
Ethanol consumption state (FS IV)
The process is defined to be in this state when ethanol is available but no sugar is in the broth, and the dissolved oxygen concentration is above the critical level. Ethanol is the only carbon source for yeast growth.
Second ethanol production state (FS V)
The conditions for this state are that both concentrations, for sugar and for dissolved oxygen, are below the corresponding critical levels. When the dissolved oxygen becomes the limiting factor for yeast growth, ethanol is produced.

According to the Zhang et al. [34], in an industrial aerobic yeast growth process where oxygen is often limited, there might exist more functional states. For instance, a state with conditions of $O_2 < O_{2crit}$ and $S > S_{crit}$, and a state with $S = 0$, $E > 0$ and $O_2 < O_{2crit}$ might be possible. Since all of experimental data came from laboratory scale cultivations, these functional states do not occur frequently [34].

A yeast growth process switches from one functional state to another when the metabolic conditions are changed. The functional state diagram of the process can be illustrated as it is shown in Fig. 4 [34].

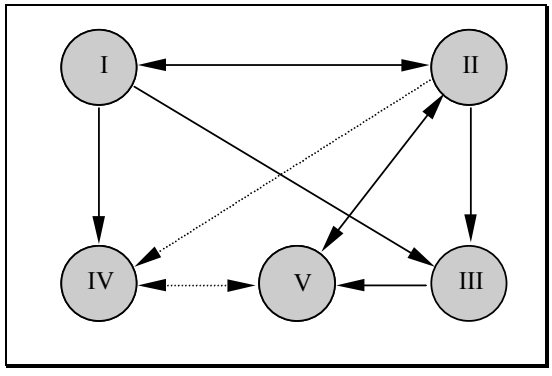

Fig. 4

In principle *FS I* can appear in all batch, fed-batch and continuous yeast growth processes. *FS IV* normally appears only in batch culture. The functional states *FS II*, *FS III* and *FS V* are normally found in fed-batch and continuous cultures. The solid arrows in Fig. 4 indicate the necessary or normal transition between various functional states of the process. The dotted arrows indicate that the transitions take place when the mode of culture changes between batch and fed-batch cultures. It should be noted that the fermentation process could be only in one functional state at any time. However, a certain functional state can appear in the process more than once during one run.

Table 1 illustrates the interrelationships of different functional states during fed-batch yeast cultivation [34]. These interrelationships will be used as rules for recognition of functional states during batch and fed-batch cultivation of yeast *S. cerevisiae.*

Table 1

Functional state	***Rules***
FS I	$S > S_{crit}$ and $O_2 \geq O_{2crit}$
FS II	$S \leq S_{crit}$ and $O_2 \geq O_{2crit}$ and $E > 0$
FS III	$S \leq S_{crit}$ and $O_2 \geq O_{2crit}$ and $E = 0$
FS IV	$S = 0$ and $O_2 \geq O_{2crit}$
FS V	$S \leq S_{crit}$ and $O_2 < O_{2crit}$

In each *FS* the process is described by a conventional type of model, called *local model*, which is valid only in this *FS*. At the second hierarchical level some numeric detection algorithms and/or rules based on expert knowledge can be used for the recognition of the *FS* and state transitions. A set of local models together with *FS* "dynamics" can be used to describe, monitor and control the overall yeast growth process.

In the first ethanol production state (*FS I*) the specific growth rate (μ) can be assumed to be constant due to dissolved oxygen limitation under high sugar concentration. The specific rate of sugar consumption (q_S) is described by Monod model. The specific ethanol production rate (q_E) is directly proportional to the difference between the specific sugar consumption rate and the critical specific sugar consumption rate according to the mass balance and the stoichiometric equation of the fermentation of sugar to ethanol. The specific dissolved oxygen consumption rate (q_{O_2}) is directly proportional to the specific growth rate, i.e. also constant. The structures of the specific rates for *FS I*, as well for all *FS,* could be seen in Table 2.

As sugar is metabolized by yeast, the sugar concentration decreases to the critical level, and the process switches from the first ethanol production state (*FS I*) to the mixed oxidative state (*FS II*). When in a batch culture, the sugar is exhausted so quickly that the mixed oxidative state (*FS II*) needs not to be considered. In practice the functional state dynamics for batch culture can be modeled so that the process switches from *FS I* directly to the ethanol consumption state (*FS IV*) when sugar decreases below the critical level. After entering *FS IV,* the yeast cells begin to synthesize the enzymes for gluconeogenesis so that cells can utilize ethanol as the carbon-source for growth. It takes some time to synthesize the induced enzymes for gluconeogenesis. This causes a lag in the yeast growth. This is also the reason for diauxic growth in the batch process. Hence in *FS IV* Monod model with a lag term can be used to describe the specific growth rate. The specific ethanol consumption rate, as well the specific

oxygen consumption rate are directly proportional to the specific growth rate, while the specific sugar consumption rate is now zero.

Table 2

Specific rate	***FS I***	***FS II***	***FS III***	***FS IV***	***FS V***
μ	μ_1	$\mu_{2S}\frac{S}{S+k_S}+$ $+\mu_{2E}\frac{E}{E+k_E}$	$\mu_3\frac{S}{S+k_S}$	$\mu_{2E}\frac{E}{E+k_E}\eta$	$\mu_3\frac{S}{S+k_S}\times$ $\times\frac{O_2}{O_2+k_{O_2}}$
q_S	$q_{sm}\frac{S}{S+k_S}$	$q_{sm}\frac{S}{S+k_S}$	$q_{sm}\frac{S}{S+k_S}$	0	$q_{sm}\frac{S}{S+k_S}$
q_E	$(q_S - q_{Scrit})Y_{E/S}$	$-q_{em}\frac{E}{E+k_E}$	0	$-\mu_{2E}\frac{E}{E+k_E}Y_{E/X}\eta$	$q_{sm}\frac{S}{S+k_S}\times$ $\times\frac{k_{O_2}}{O_2+k_{O_2}}Y_{S/E}$
q_{O_2}	$q_{O_2 1}$	$q_E Y_{O_2/E}+$ $+q_S Y_{O_2/S}$	$q_{O_2 3}\frac{S}{S+k_S}$	$\mu_{2E}\frac{E}{E+k_E}Y_{O_2/E}\eta$	$q_{O_2 3}\frac{S}{S+k_S}\times$ $\times\frac{O_2}{O_2+k_{O_2}}$

The yeast growth process enters the mixed oxidative state (*FS II*), when the sugar concentration in the broth declines below the critical level in fed-batch or continuous culture. In this state, both sugar and ethanol are cometabolized to produce energy and the intermediates for yeast growth. The specific growth rate is expressed accordingly as a sum of two terms, one describing the contribution of sugar and another – the contribution of ethanol to yeast growth. Both terms have the structure of Monod model. Monod model is also used for the specific ethanol and sugar consumption rates. The specific oxygen consumption rate is naturally obtained by a summation over two terms, which are directly proportional to the specific sugar consumption rate and the specific ethanol production rate, respectively. When the ethanol is depleted and the sugar concentration is below the critical level, the yeast growth process enters the complete sugar oxidative state (*FS III*). The sugar is the only carbon source in the broth and the limiting factor for growth as well. In *FS III* the specific rates of yeast growth, sugar utilization and oxygen consumption are described by Monod models. The process enters the second ethanol production state (*FS V*) when the dissolved oxygen decreases below its critical level. The specific rates of yeast growth and oxygen consumption depend on the dissolved oxygen concentration and the sugar concentration. These rates are assumed to have the same structures as in *FS III* except that the terms are multiplied with a term dependent on the dissolved oxygen concentration. The specific rate of sugar consumption has the same structure as in the previous states. The specific rate for ethanol production is directly proportional to the specific rate of sugar consumption with a factor of oxygen limitation. Table 2 summarizes the structures of the specific rates of the local models in all functional states [34].

State decomposition of *Escherichia coli* cultivation

In this section it will be presented that, the concept of functional state modelling, originally developed by Zhang [34] for yeast cultivation processes, could be applied for modelling of *Escherichia coli* cultivation. Based on the many research reports about the changes in *E. coli* process behaviour during different cultivation conditions (high or low glucose concentrations, oxygen limitation or oxygen starvation, etc.) it is evident that there are a lot of analogies between the yeast and *Escherichia coli* metabolisms. Some of investigations, substantiated this statement, are shortly presented below.

E. coli has a similar behaviour in comparison to yeast. Both yeast and *E. coli* can undergo aerobic and anaerobic metabolism. Both in yeast and *E. coli* aerobic metabolism results in the production of CO_2 and water. Yeast anaerobic metabolism results in the production of ethanol, while *E. coli* anaerobically produces acetate. Many types of yeast produce and utilize ethanol under aerobic conditions [26, 34]. This fact is taken into account in the Zhang local models proposed to functional state modelling of yeast. At the same time, it is known that *E. coli* can also synthesize a significant amount of acetate and utilize the acetate during the growth of *E. coli* on glucose under aerobic conditions [2, 11, 12, 19, 20, 33]. In the presence of a glucose feed *E. coli* utilize acetate 3 times faster than in the absence of glucose [32]. Acetate has a critical role as it functions as both a product and a reactant [22]. Together with its counterpart phenomenon in yeast, i.e., the aerobic ethanol production, this process is now known as glucose overflow metabolism [7, 9, 13, 21, 23, 31, 32]. High specific growth rate, high specific glucose uptake rate, bottlenecks in the Krebs cycle, limited respiratory capacity, or a combination of any of the above factors has been suggested to trigger acetate or ethanol overflow metabolism.

The metabolic flux model of *E. coli* is presented in Fig. 5. As it can be seen the model is identical to the *S. cerevisiae* one, shown in Fig. 3.

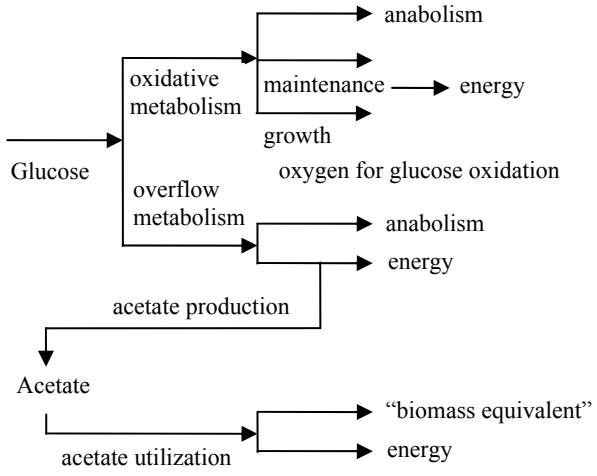

Fig. 5

When *E. coli* strains are grown aerobically to high cell densities on glucose, two fundamental phenomena occur. There are two distinct metabolic routes that lead to acetate formation in *E. coli* fermentations. The first of these concerns overflow metabolism and occurs at high specific growth rates under oxygen excess conditions, where the overabundant supply of energy results in acetate excretion. Acetate is produced when carbon flux into the central

metabolic pathway exceeds the biosynthetic demands and the capacity for energy generation within the cell. [14, 31, 32]. The second phenomenon occurs as a result of either oxygen limitation or oxygen starvation, where glucose metabolism occurs via the mixed acid fermentation, with acetate again being one of the products [3, 4, 5, 14, 15, 31].

In general, fermentative metabolism is not the same in all microorganisms, but there are many similarities. Nielsen and Villadsen [18] have illustrated the analogy in the fermentative metabolism of yeast *S. cerevisiae* and bacteria *E. coli*, presented respectively in Fig. 6 and Fig. 7 [24].

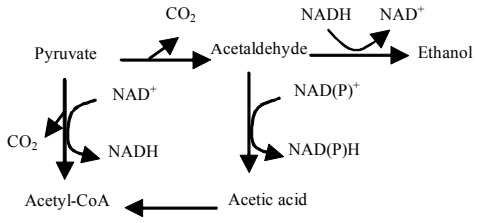

Fig. 6

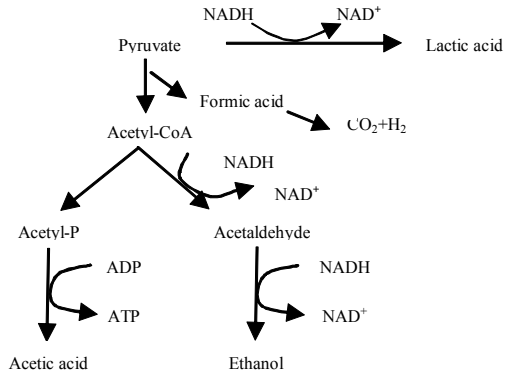

Fig. 7

Based on foregoing discussion and Zhang investigations for yeast [34], one possible division into different functional states according to cultivation conditions (glucose concentration value, oxygen concentration value, acetate production or acetate utilization) is proposed here. Due to proved similarities between yeast and *E. coli* metabolisms, Table 3 presents the rules for recognition of functional states during fed-batch cultivation of *E. coli.* Acetate consumption state (*FS IV*), characterized only batch culture, is omitted because fed-batch mode is here considered.

Table 3

Functional state	***Rules***
FS I	$S > S_{crit}$ and $O_2 \geq O_{2crit}$
FS II	$S \leq S_{crit}$ and $O_2 \geq O_{2crit}$ and $A > 0$
FS III	$S \leq S_{crit}$ and $O_2 \geq O_{2crit}$ and $A = 0$
FS V	$S < S_{crit}$ and $O_2 < O_{2crit}$

The description of different functional states for *E. coli* cultivation process is the same as one for yeast, with the remark that acetate is here considered instead of ethanol. Based on the rules presented in Table 3 the state decomposition process could be applied to fed-batch fermentations of *E. coli*.

Conclusion

Taking into account all application of multiple-model approach for fermentation processes, as more convenient for further process control is functional state modelling approach. The approach is originally developed by Zhang et al. [34] for aerobic yeast growth process. The application of such approach is appropriate because of two reasons. First, the definition of the different functional states is possible because of well known mechanisms in aerobic yeast growth process. Second, defined functional states could be comparatively easily recognized, based on on-line measurements of substrate (glucose) and dissolved oxygen concentrations. Known analogies between fermentation metabolisms of yeast and *E. coli* allow functional state modelling approach to be applied for *E. coli* cultivation as well. The concept implementation leads to the process description with simpler and more transparent local models that help to understand better the process behavior and to simplify the process modelling.

Acknowledgements

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